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Cover photo: A female brown widow spider, *Latrodectus geometricus* (Theridiidae), with her own egg sacs, and an assortment of egg sacs of other spiders found in central California (see page 176). Photos by Rick Vetter.

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REVIEW

Spider locomotion on the water surface: biomechanics and diversity

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Abstract. Spiders in many families are capable of locomotion on the surface of water, a capability that, at its simplest, requires only a strongly hydrophobic integument and the same postures and motions that are used on land. Specialized aquatic gaits, in contrast, are only characteristic in the Pisauridae, Trechaleidae, Ctenidae, and Tetragnathidae. They are less common features of aquatic locomotion in Lycosidae, are only occasionally encountered in Salticidae, and are rare in Araneidae. Most of what is known about the biomechanics of these specialized gaits comes from research on fishing spiders (Pisauridae) and, because the physics and hydrodynamics are similar in many respects, on water striders (Insecta: Hemiptera: Gerridae). In what follows, I have concentrated on the biomechanics of propulsion in water-walking spiders and water striders beeause propulsion on the air-water interface was mysterious until the 1990s when researchers began seeking answers to the central question: What provides the resistance against which a spider or water strider pushes when it sweeps its legs backward? The answers, now nearly complete, include a) dimple distortion, b) drag, c) generation of vortices, and d) nanoscale brushing of the water surface by hydrophilic hairs.

Keywords: Aquatic propulsion, air-water interface, hydrophobic surface, nanoscale, gait, rowing, galloping, phylogeny, performance, fishing spiders, water striders

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1. INTRODUCTION

The surface of a pond or stream is an interface between air and water. The surface itself is in tension and thus is capable of supporting an organism that is denser than the underlying water. And the underlying water, being both dense and somewhat viscous, resists local changes in its momentum, making it possible for an organism to push against the water and thus propel itself (Denny 1993; Vogel 1994).

A number of organisms make use of this peculiar environment, some just passing through the way an aquatic mite does when quitting its submerged existence (Meyer 1985) and others, like water boatmen (Hemiptera: Corixidae: Hinton 1976), refreshing the oxygen they use in plastron respiration (Flynn & Bush 2008). For some, though, like water striders and fishing spiders (Fig. 1), the interface is their primary (if not necessarily their obligate) physical substrate, supporting communication, predation, and locomotion (Wilcox 1979a, b; Foelix 2011).

My purpose in the following pages is to consider walking on water by arthropods, particularly spiders but also water striders (Heteroptera: Gerridae), paying attention throughout to the relationships between structure and function.

2. PERFORMANCE

2.1 Gaits.—Spiders in a wide variety of families are capable of effective locomotion on the water surface (Ehlers 1939; Shultz 1987; Barnes and Barth 1991; Suter et al. 2003; Stratton et al. 2004). This locomotion can take the form of walking or running with the same stepping gait as is seen on solid ground (Shultz 1987) or can involve altogether different gaits, presumably honed by natural selection, that function effectively on the water surface. Three qualitatively distinct water-surface gaits have been identified (Table 1).

Thus there are really four major gaits used by spiders on the water surface: 1) walking with the alternating tetrapod

Figure 1.—*Dolomedes triton*, a fishing spider (Pisauridae), and *Gerris* sp., a water strider, at rest on the water surface. The spider's weight is borne primarily by the distal parts of its legs where they push down on the water surface forming visible dimples there. The ventral surface of the prosoma also presses on the water surface as indicated by the dimple under that part of the spider. The water strider's weight is borne entirely on the ends of its six legs.

locomotion that is characteristie of spiders on solid substrates, 2) rowing, using pairs of contralateral legs in synchrony, 3) crawling, using the first pair of legs in alternation and 4) galloping, in which downward and backward thrusts of the first three pairs of legs produce strings of leaps from the water surface. Videos of the aquatic locomotion of a rowing and galloping *Dolomedes triton* (Walckenaer, 1837), a crawling *Tetragnatha* spp., a rowing salticid, and a walking *Geolycosa rogersi* Wallace, 1942 (a lyeosid that does not have a gait specialized for use on the water surface), are available online at http://www.bioone.org/doi/suppl/10.1636/M13-14.

2.2 Velocity.—The velocity of water-surface locomotion has been measured in only a few species of spiders, with a concentration on the nursery web or fishing spiders (Pisauridae). Suter et al. (2003) compared gaits and velocities of spiders in seven families (Fig. 2), finding that galloping fishing spiders (Pisauridae) had by far the highest absolute velocities (mean > 0.4 m/s), but that among spiders with gaits that had no aerial phase, *Tetragnatha* sp. (Tetragnathidae) using the "crawl" achieved both the highest absolute and relative velocities (0.25 m/s, 29 body lengths/s). Hu and Bush (2010) reported fishing spiders rowing at a mean velocity of 0.15 m/s and galloping at 0.35 m/s.

Suter and Gruenwald (2000a) measured rowing velocities achieved by many sizes of the fishing spider, *Dolomedes triton*: spiders spanning a 600-fold range of masses could all row at about the same velocity (mean = 0.11 m/s), with the largest variation occurring at the very smallest sizes. In terms of relative velocity, however, largely because of the increase in stride frequency with decreasing mass, the smallest spiders rowed at about 42 body lengths per second while the largest spiders only achieved about 6 bl/s.

2.3 Efficiency.—For most spiders that frequent the water surface, we lack estimates of the efficiency of the gaits used (but see Brown & Formanowicz 2012). However, in *Dolomedes* (Pisauridae), Hu and Bush (2010) calculated locomotion efficiency using the Strouhal number (*St*), the dimensionless ratio of the product of stroke frequency and stroke amplitude to forward velocity, as 0.3 for rowing and 0.4 for galloping. These are close to what is found for swimming in fish of all sizes (0.25 to 0.35; Denny 1993; Vogel 1994, 2013), for the fastest water-walking insects, water striders (Gerridae, 0.2; Hu and Bush 2010) and for birds in flapping flight (Taylor et al. 2003). Taylor et al. (2003) have argued that natural selection on fluid-based locomotion efficiency (i.e., in air and water) has constrained animals to the range of *St* expected for high propulsive efficiency, $0.2 < St < 0.4$.

More interesting in the current context would be comparisons of locomotion efficiency in spiders of about the same size that use a terrestrial gait on water vs. ones that use a specialized aquatic gait. This would be readily possible between selected members of Lyeosidae and any of the Pisauridae (Stratton et al. 2004).

3. PHYLOGENETIC DISTRIBUTION

Locomotion on the water surface, supported there not by buoyancy but by surface tension or by hydrodynamics, is uncommon but has apparently evolved independently many times in the animal kingdom. Bush and Hu (2006) count more than 1200 species that either habitually or in extremis propel themselves aeross the water surface, including mammals, birds, reptiles, fish, insects and spiders. Among spiders, the fishing spiders (Pisauridae) (Fig. 1) are best known and may be the most adept at this form of locomotion (below), but they are by no means alone.

In the most extensive compilation of information on water-surface locomotion in spiders, Stratton et al. (2004) surveyed the eapabilities of nearly 250 spider speeies in 42 families. In each species, they looked both at the interaction between the water surface and the spider's integument, where wettability (see 4.1.2) determines whether the spider is trapped by the water's adhesive properties or can exploit the water's surface tension, and at the spider's propulsive behavior, if any. They found 1) that many spiders had hydrophilic surfaces, either in part or in full, that rendered them incapable of escaping

Table 1.—Specialized gaits used by spiders on the water surface.

Name	Exemplar	Description	Notes	References
Row	*Dolomedes* (Pisauridae)	legs III and II (or II nd I for Araneidae), in that order, provide rowing propulsion, members of each pair sweeping in unison; stroke primarily in lateral plane and posteriad; continuous contact between spider and water surface; (named by analogy with the propulsion of rowboats)	characteristic in Pisauridae, Trechaleidae, Ctenidae; characteristic in some species in Lycosidae; occasional in Salticidae; rare in Araneidae; variants include legs I in propulsion	Barnes & Barth 1991; Suter et al. 2003; Stratton et al. 2004
Gallop	*Dolomedes* (Pisauridae)	legs III, II, and I, in synchrony; stroke primarily in vertical plane and posteriad; contact between spider and water only during power stroke; (named by analogy with the galloping of horses and other mammals)	characteristic in Pisauridae	Gorb & Barth 1994; Suter & Wildman 1999
Crawl	*Tetragnatha* (Tetragnathidae)	legs I, alternating, sweep to the side and posteriad; (named by analogy with the human crawl swimming stroke)	known only in Tetragnathidae where it is characteristic in many species	Suter et al. 2003; Stratton et al. 2004

water's adhesive attraction, but 2) that the remainder had largely hydrophobic surfaces and so were well supported by the water surface. Among those species that stayed dry and on top of the air-water interface, a few remained immobile unless prodded while most immediately moved away using gaits that varied between ungainly scrambling and coordinated walking, rowing, crawling, or galloping.

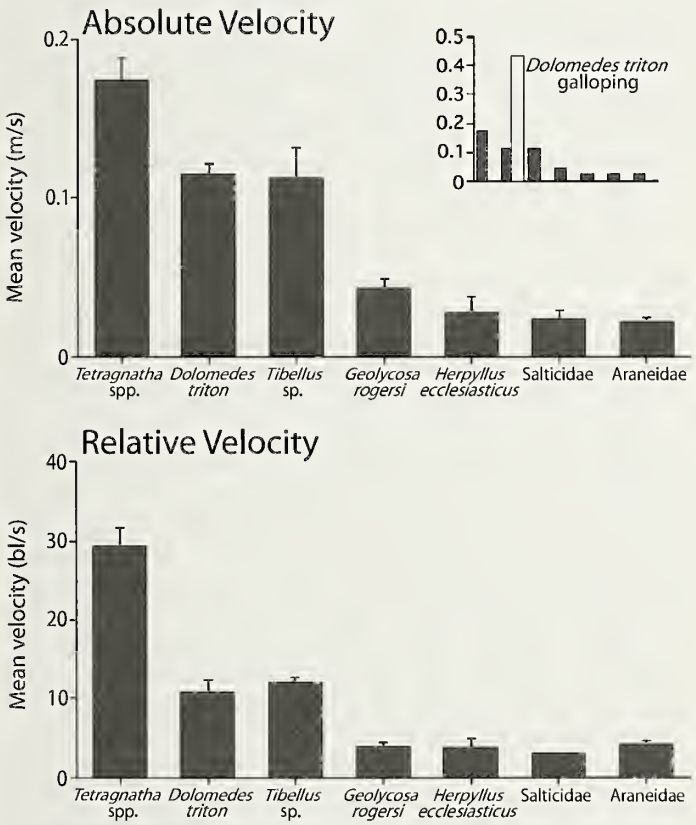

Figure 2.—Absolute and relative mean velocities of crawling, rowing or walking spiders, and galloping spiders (inset). By both absolute and relative (body lengths per second) measures, the long-jawed orb weavers (crawling; Tetragnathidae) were fastest. Velocities of *Dolomedes triton* (rowing; Pisauridae) and *Tibellus* sp. (walking; Philodromidae) were intermediate by both measures, but *D. triton* surpassed all others when galloping (inset). Adapted from Suter et al. 2003.

Although rowing, the gait on which Stratton et al. (2004) focused, was performed by a few orb weaving spiders (Araneidae) and a few jumping spiders (Salticidae), most of the rowing spiders were in the Grate-Shaped Tapetum clade (GST; Silva Davila 2003) (Fig. 3). There, rowing was found only in Lycosidae, Pisauridae, Trechaleidae, and Ctenidae. Stratton et al. (2004) argued that rowing on the water surface evolved four times in spiders: once in jumping spiders, once in orb-weavers, once in the branch of the lycosoids that includes Lycosidae, Pisauridae, and Trechaleidae, and once in Ctenidae. Their analysis also included the juxtaposition of rowing propensity and characteristic habitat—their conclusion in that realm was that "phylogeny is a stronger force than current selection pressures arising from habitat in determining whether members of a species are capable of rowing" (Stratton et al. 2004:72).

4. BIOMECHANICS OF LOCOMOTION

Locomotion on the water surface presents animals with two core problems. On land, the solid surface resists the downward push that gravity imparts to denser-than-air organisms, whereas the density and viscosity of water offer much less resistance to the downward push. Similarly, on land, macroscopically irregular surfaces and static friction oppose leg motions in the horizontal plane, allowing an organism to push itself forward, while on water friction/drag can be very low and any horizontal leg motions meet with comparatively little resistance. These constitute the support and propulsion problems, respectively. We will consider these separately even though, for spiders and other surface-dwelling arthropods, solutions to the two problems share the exploitation of surface tension.

4.1 Support.—When the fishing spider, *Dolomedes triton* (Pisauridae), is at rest on the water surface, its weight distorts the surface, forming "dimples" wherever the spider and the water meet (Fig. 1). The spider remains at the surface because the downward push of its weight is opposed by a combination of the upward push of surface tension, accounting for about two-thirds of the spider's weight, and the upward push of

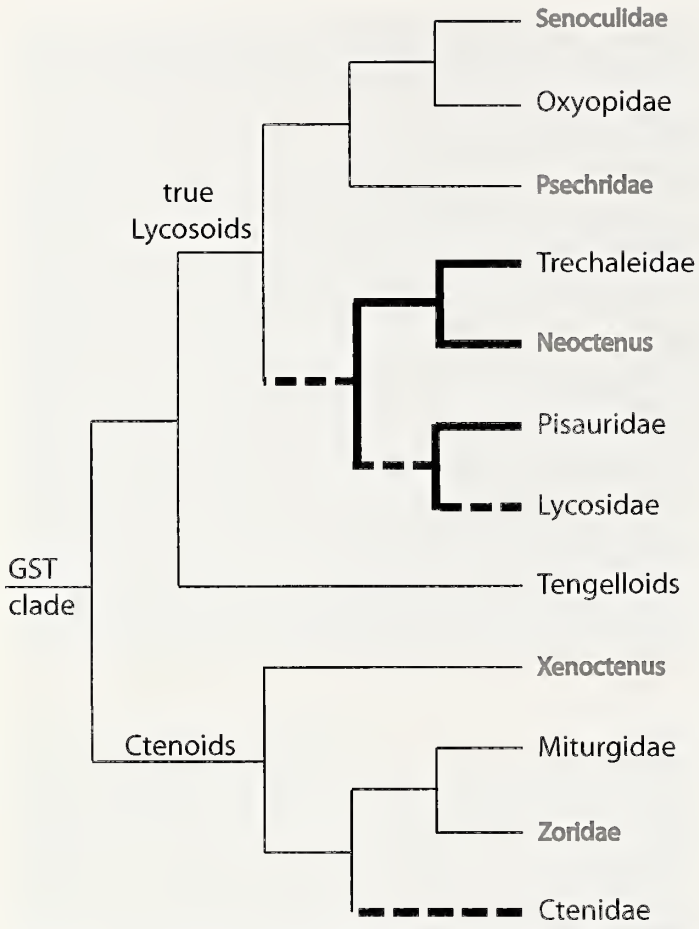

Figure 3.—A portion of the phylogenetic tree of spiders, showing the Grate-Shaped Tapetum clade that contains most of the spiders that use rowing as their characteristic gait during aquatic locomotion. Adapted from Stratton et al. 2004.

buoyancy accounting for the remaining one-third (Hu & Bush 2010). (This is one respect in which fishing spiders and water striders differ quantitatively: the water striders, being less massive and having comparatively thin legs, are supported almost entirely by surface tension, with buoyancy being negligible as a supporting force.) The legs and the undersides of the cephalothorax and abdomen remain dry (Stratton & Suter 2009). This relationship between nonwettable body parts and the malleable surface of water must be central in any discussion of water surface locomotion.

4.1.1 Properties of the water surface: The attributes of the air-water interface and their implications for organisms are clearly delineated in two well-known texts, *Air and Water: The Biology and Physics of Life's Media* (Denny 1993) and *Life in Moving Fluids: The Physical Biology of Flow* (Vogel 1994), and in a more recent review of locomotion on the water surface (Bush and Hu 2006).

The interface between water and air has a peculiar structure. Because water molecules are polar they are attracted to each other, giving water cohesion. The absence of comparable molecular interactions between water molecules and air molecules, that is, the absence of adhesion, means that the interface between the two fluids is relatively stable. Importantly for the current discussion, the cohesion between water molecules at the interface puts the water surface in tension, measured in units of force per distance (usually N/m). It takes energy or work to increase the exposed surface of the water, which means that the surface tension resists deformations such as those caused, for example, by the leg of a spider pushing down on the surface, or by any small wave or ripple.

In the context of support on the water surface, the air-water interface can be breached in two ways. If an appendage or other body part has a strongly hydrophilic surface, the water's surface tension provides no resistance and the appendage penetrates the surface unimpeded; if the part's surface is strongly hydrophobie, however, it eannot become submerged unless the downward foree on it exceeds the upward resistance of the surface tension as it is applied along the perimeter of the contact area between the body part and the water surface. The degree to which these generalizations are true depends on the physical and chemical properties of the surface of the appendage (Bush et al. 2008).

4.1.2 Properties of spider surfaces: The species of spiders that most successfully exploit the water surface for locomotion have integuments rich in hairs that are themselves strongly hydrophobic (Suter et al. 2004; Stratton and Suter 2009; Foelix 2011). This coincidence of surface roughness (hairs, but also nano- and micro-scale unevenness) and molecular-level hydrophobicity (e.g., cuticular waxes) makes any surface impressively resistant to wetting (Wenzel 1936; Cassie and Baxter 1944; Quéré 2002, 2008). The two properties have been combined many times throughout biological evolution and are known to confer strong water repellency on both plants (Neinhuis and Barthlott 1997; Cerman et al. 2009) and animals, including insects (Holdgate 1955; Neville 1975; Gao and Jiang 2004) and spiders.

In the preceding two paragraphs, "hydrophobic" and "hydrophilic" appear to be the two parts of a dichotomy, whereas in fact they are categorical names for regions on a continuum. The continuum is defined by the ratio of the cohesion energy of water (its molecules' tendency to attract each other) and the adhesion energy where the water touches the solid substrate (the tendency of the water and solid molecules to stick together). Measuring the energies of cohesion and adhesion is difficult, but their ratio is easily measured because, as the ratio changes, so does the contact angle between the water and the solid surface (Young 1805; Denny 1993; Vella 2005; Bush et al. 2008). Water-walking arthropods have leg surfaces with contact angles that exceed 90°, above which the leg surface is nominally hydrophobic. The fishing spiders (147°, Stratton et al. 2004) and water striders (168°, Gao & Jiang 2004) have surfaces that are close to or above 150°, at which point they are often referred to as superhydrophobic (Bush et al. 2008).

Bush and his colleagues (Bush et al. 2008) summarized the interactions between arthropod integuments and water with a very useful combination of diagrams, photographs and mathematical models to augment their analyses. Because their review is both pertinent and nearly complete, it should be consulted by those interested in a more detailed summary than I have given above. More recently, Prakash & Bush (2011) showed that the orientation of surface roughness causes anisotropy (directionality) in the interactions of water and arthropod surfaces, an asymmetry that may be most

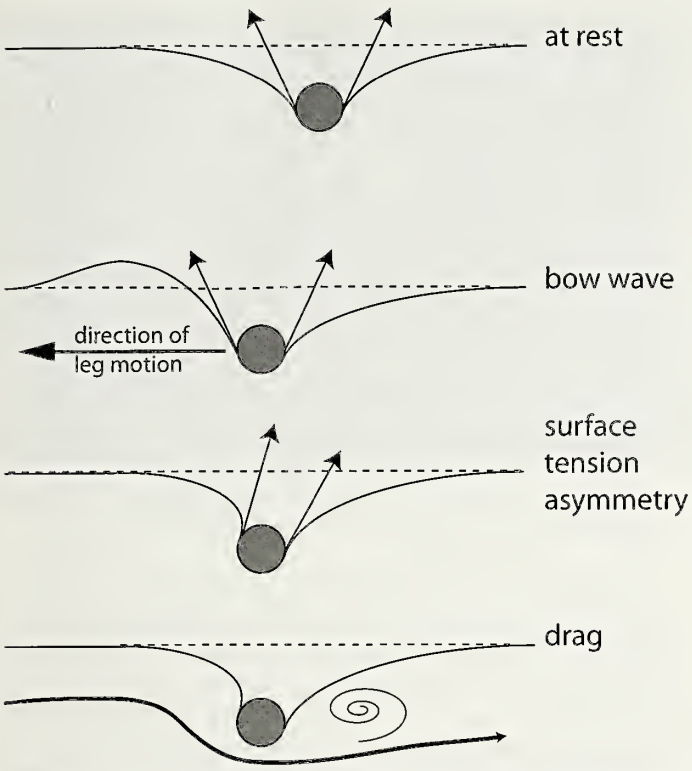

Figure 4.—Schematic representations of the relationship between the spider's leg (in cross section) and the dimple at rest (top) and during a rowing power stroke (bottom three). At rest, the curvatures of the sides of the dimple are symmetrical as are the directions of the vectors representing the forees on the leg due to surface tension. During a rowing power stroke, one or more of the three hypothesized sources of resistance to the leg's motion could be in play, as could brushing the water surface with hydrophilic hairs (not shown; see Hu & Bush 2010). Modified from Suter et al. 1997.

important for water-surface locomotion by very small spiders and water striders (see 4.2.1).

Having a strongly hydrophobic, hairy surface not only contributes to water-surface locomotion but also makes possible the physical lung with which submerged insects and arachnids can breathe (Crisp and Thorp 1948; Hinton 1976; Rovner 1986; Hebets and Chapman 2000; Flynn and Bush 2008; Balmert et al. 2011). Recent work confirms that the combination of hydrophobicity with hairiness or nanoscale roughness also renders a surface self-cleaning (Barthlott and Neinhuis 1997; Neinhuis and Barthlott 1997; Hiller 2009). In many spiders, this may currently be only one of its functions (Stratton and Suter 2009), but because hairy hydrophobicity was apparently a pre-adaptation to effective water-surface locomotion, its ancestral self-cleaning function may have been central.

4.2 Propulsion.—Spider locomotion on the water surface, like the locomotion of the better known water striders (Insecta: Gerridae), was well described before the mid-1990s but was not well explained; that is, the kinematics had been described (McAlister 1959; Anderson 1976; Bowdan 1978; Shultz 1987; Barnes and Barth 1991; Gorb and Barth 1994), but the mechanism of transfer of momentum between the spider or strider and the water, which must accompany propulsion (satisfying the law of conservation of momentum: Dickinson et al. 2000), was not known. [Although insects and spiders are not particularly closely related (Regier et al. 2010), it is likely that their water-surface locomotion can be understood in many of the same ways, at least concerning rowing.]

4.2.1 Rowing: With respect to rowing, two hypotheses were in play in the mid-1990s (Fig. 4), neither necessarily to the exclusion of the other. Denny (1993) noted that any object moving at the water surface must create a bow wave if the object's velocity exceeds about 0.23 m/s, the lowest speed at which a wave, responding to the restorative forces of gravity and capillarity, can move. He reasoned that a strider's leg tips, moving backward faster than the wave minimum, would produce a bow wave against which it would push or gain purchase (Fig. 4, bow wave). The wave's sternward motion would contain the sought for momentum corresponding to the strider's forward motion.

Vogel (1994) proposed, in contrast, that the resistance provided by the water arises out of a distortion of the dimple's shape—when the leg is at rest, the dimple's net resistance is vertical, but when the leg is in motion, the associated dimple's leading (toward the strider's posterior) surface is more strongly curved than its trailing surface, and the net surface tension resistance points toward the spider's anterior. This hypothesis did not directly address the question of momentum transfer, but it did reflect the certainty that, whatever else was happening, the points of contact between spider or strider and the water surface were located at the cusp of the dimple. Thus, an asymmetry in the surface tension vectors had to be part of the explanation (Fig. 4, surface tension asymmetry).

Three years later, Suter et al. (1997) reported on a series of experiments with fishing spiders, *Dolomedes triton*, that revealed the following: a) as leg velocity increased in the range of 0 to 0.2 m/s, the drag caused by water flowing around the leg and its attached dimple increased rapidly, providing on the order of 85% of the resistance force experienced by the leg; and b) as surface tension was experimentally decreased, small changes in resistance forces were detected, indicating a persistent but still secondary role of surface tension in horizontal propulsion. From a), it followed that a bow wave need not be present to allow water-surface propulsion, a conclusion that removed the paradox identified by Denny (1993, 2004), namely, how could juvenile water striders (and fishing spiders), with leg tip velocities consistently < 0.23 m/s, propel themselves across the water surface in the absence of bow waves? Drag appeared to be the answer. [A core premise of Denny's paradox (Denny 1993, 2004), that waves cannot be generated by objects at the air-water interface that are moving at velocities < 0.23 m/s, has since been falsified for objects moving impulsively or in arcs or circles, as do the propulsive legs of spiders and water striders (Bühler 2007; Chepelianskii et al. 2008; Closa et al. 2010).]

Thanks to further work by Bush and Hu and their colleagues (Hu et al. 2003; Bush and Hu 2006; Hu and Bush 2010) and to Gao and Feng (2011), on water-surface locomotion in water striders and others, we have a nearly complete picture that is both theoretically and empirically grounded.

When an adult water strider or fishing spider sweeps its propulsive legs backward, usually first shifting its weight so

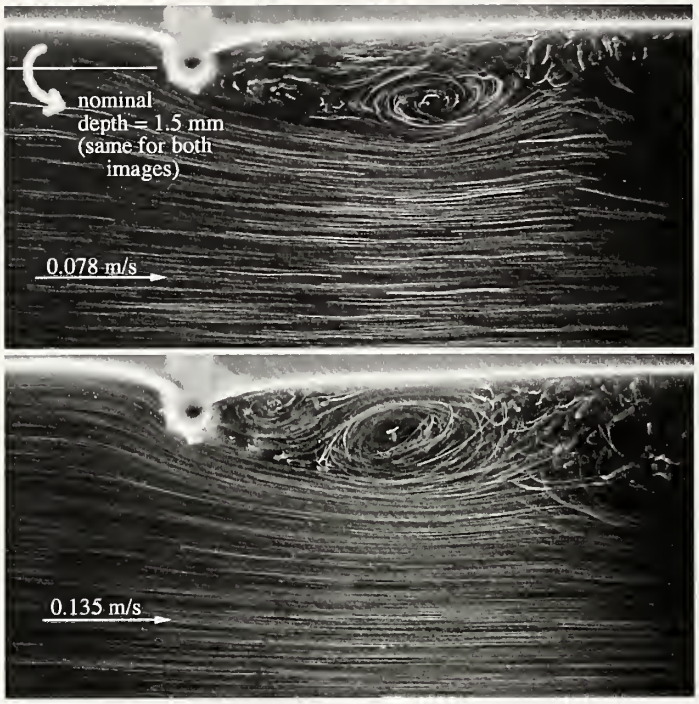

Figure 5.—Particle image velocimetry (PIV) revealing the motions of water during a steady state experiment in which water at constant velocity flowed past a stationary hydrophobic model of a fishing spider's leg. Especially in the lower panel, both the bow-wave and the dimple asymmetry are visible. In both panels, vortices can be seen forming and being shed downstream of the model leg.

that the weight is borne primarily by the propulsive legs, the accompanying dimples deform. The deformation is of the form depicted in Figs. 4 & 5 and results in a resistive force, due to surface tension, that has a net forward-pointing horizontal component. This force opposes the backward motion of the leg and provides the purchase needed for the spider to achieve forward motion. The resistive force is applied to the spider's leg along the perimeter of the area of interaction between the leg and the water surface. Recent experiments have revealed that bending of the long, slender legs of water striders can alter the biomechanical interaction of the leg as it forms a dimple in the water surface (Ji et al. 2012), a detail that may be less important in fishing spiders because of their proportionately stiffer legs.

The cause of the dimple's deformation, in turn, is the water's mass- and viscosity-related resistance to flow, with the mass-related resistance being predominant (Gao & Feng 2011). But the flow does happen, producing a wave at the leading surface of the dimple and vortices under the trailing surface (Fig. 5)—these waves and vortices account (literally) for the momentum transfer between the animal and the water (Rinoshika 2012).

A second resistive force is also operative in these adult surface-walking animals as well as in their smallest progeny, a force neither anticipated in the earlier models of Denny (1993) and Vogel (1994) nor in the studies of Suter and his colleagues (Suter et al. 1997; Suter and Wildman 1999; Suter & Gruenwald 2000a). It is now clear that nanoscale interactions between the strider's leg hairs and the water surface (Feng et al. 2007) have magnitudes large enough to contribute

Figure 6.—A fishing spider during galloping, photographed just as its legs were leaving the water after a power stroke.

substantially to the resistance experienced when the animal sweeps its legs backwards, "brushing" the water surface (Hu and Bush 2010). Moreover, this "brushing" is anisotropic, meaning that it is directionally asymmetrical. With hydrophobic hairs anchored proximally on a propulsive leg and lying approximately parallel to the leg's surface, the leg meets more resistance when the net direetion of water motion is up the leg, and meets less resistance in the opposite direction (Prakash & Bush 2011). This anisotropic "brushing" also makes it less energetieally costly to pull the leg from the water surface at the end of a rowing stroke (Prakash & Bush 2011) or at the end of a galloping stroke (see 4.2.2). This "brushing," in both its isotropic and anisotropic forms, is likely to be the only resistance force available to newly hatched water striders and fishing spiders.

For a comprehensive but still compact treatment of the issues outlined above, see Hu and Bush (2010). To explore an elegant two-dimensional finite-element simulation of water-surface propulsion by small arthropods, one that largely confirms the work described above, see Gao and Feng (2011).

4.2.2 Galloping: In contrast to rowing, the galloping gait commonly seen in pisaurid spiders (Fig. 6) may not have an equivalent in water striders or other insects, but it does have an analog in reptiles. The basilisk lizard, *Basilicus basilicus* (Corytophanidae), runs across the water surface using what has been called a slap and stroke gait (Glasheen and McMahon 1996a, b; Hsieh 2003; Hsieh and Lauder 2004). This involves pushing each hind foot downward fast enough to sharply impact the water surface (the slap), then following through and thereby transitorily opening an air-filled cavity in the water (the stroke), and finally withdrawing its foot before the cavity collapses.

A fishing spider also uses a downward and then backward stroke (Gorb and Barth 1994; Suter and Wildman 1999; Hu and Bush 2010), which briefly opens an air-filled cavity, and then withdraws its legs in preparation for the subsequent stride. The hydrophobicity of the spider's integument (see 4.1.2), however, means that the resistance to the "slap" has both inertial and surface tension eomponents (Hu and Bush 2010), whereas in the lizard, resistance to the slap is entirely inertial [this sets the spiders apart from the lizards with respect

to their inclusion in a class of processes called Froude mechanisms (Aristoff et al. 2011), a subject that is interesting but is beyond the scope of this review]. In both cases, the slap phase and the first part of the stroke phase do the work against gravity, elevating the animal, and the latter part of the stroke phase provides the horizontal propulsion (Glasheen and McMahon 1996a; Suter and Wildman 1999; Hu and Bush 2010).

At the end of bouts of rowing and galloping, water-walking spiders are slowed by the same forces that offered resistance to the propulsive strokes that got them moving in the first place — the horizontal component of the net surface tension vector due to dimple deformation, and the micro-scale drag forces encountered as the spiders' leg and body surfaces brush the water surface (see 4.2.1). In addition, during shore-initiated predatory attempts, fishing spiders may grasp their own dragline silk, previously anchored to solid substrate on shore, thereby bringing themselves to a rapid stop (Gorb and Barth 1994).

4.2.3 Jumping & sailing: Spiders that frequent the water surface sometimes take advantage of its peculiar properties (see 3.1.1) in ways that do not quite fit the definition of active locomotion but should be mentioned here nevertheless: jumping and sailing.

The vertical jumps from the water surface performed by fishing spiders (Suter and Gruenwald 2000b; Suter 2003; Hu and Bush 2010), and probably by other lycosoid spiders, involve all eight legs. The spider, which is initially splayed on the water surface, simultaneously and forcefully depresses its legs, producing with each leg the same kind of slap and stroke motion that is characteristic of the spider's legs during galloping. In this case, though, the horizontal forces caused by the legs' movements approximately cancel each other, resulting in a vertical leap but little horizontal displacement.

In the contexts of function and fitness, these vertical jumps are unlikely to reduce mortality due to attacks by fish from below (Suter and Gruenwald 2000b), but are quite effective in evasion of attacks from the side by frogs (Suter 2003). In the latter situation, the necessary and sufficient trigger for the vertical jump was shown to be the compression wave front that precedes the attacking frog, detected by the spiders' leg-borne trichobothria. Attacking spiders, either on the water surface or on land, also are preceded by that kind of compression wave front, potentially rendering them detectable by their intended prey (Casas et al. 2008).

As discussed above, spiders with strongly hydrophobic surfaces, at rest, have a relatively tenuous physical contact with the water surface. This presents problems for active locomotion but facilitates passive displacement when a breeze is present. An entirely passive (prone, legs and body in contact with the water surface) water-borne spider would experience some air movement close to the water, but would be pushed along relatively slowly. This is because air velocity at the surface, at the base of the local boundary layer, would be substantially lower than the velocity just a few millimeters above that (Denny 1993).

Fishing spiders, and perhaps other lycosoid spiders, often either elevate their forelegs (Deshefey 1981) or raise their bodies by standing on the tips of their tarsi (Suter 1999) when on the water surface in a breeze. These postures, called sailing, take advantage of the increased air velocities found higher up in the boundary layer. However, as is the case with ballooning (e.g., Reynolds et al. 2006), there appears to be little opportunity for sailing spiders to affect their direction of motion.

5. SUMMARY

Many spiders have hydrophobic surfaces that allow them to remain dry on the surface of a pond or stream. Among these, some can achieve coordinated and effective locomotion on that air-water interface despite their tenuous contact with it. A few groups of spiders have evolved specialized gaits, different from those used on solid substrates, that appear to be adaptations to locomotion on the water surface.

Empirical and theoretical research during the last two decades has revealed many of the morphological, biomechanical and fluid dynamic components of the rowing locomotion that allow some spiders, like fishing spiders, and some insects, like water striders, to inhabit that habitat preferentially. The central questions addressed by the research have concerned how water-walking arthropods achieve propulsion. The answers are interesting: 1) when at rest on the water surface, a spider's or water strider's weight distorts the water surface, forming a dimple wherever the weight is borne; 2) the backward sweep of a leg and its dimple distorts the dimple, thereby causing the sum of the two vectors of surface tension (one along the leading edge of a leg, the other along the following edge) to have a forward component, thus offering resistance to the animal's backward push; 3) at the same time, the leg-cum-dimple acts as an oar blade, pushing water backward and the animal forward; 4) also at the same time, and probably especially important for early instar fishing spiders and water striders, hydrophilic parts of the ventral surfaces of the legs (not yet demonstrated in spiders) are in intimate contact with the water surface, and their backward motion encounters drag resistance; and 5) the forward momentum that the arthropod achieves is matched by the momentum of backward-moving vortices of water. To some extent, these same components are operative in the less common forms of water locomotion that include galloping, jumping and sailing.

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Hogna radiata males do not deplete their sperm in a single mating

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Abstract. To the extent that sperm production or mating opportunities are limited, males are expected to allocate their sperm optimally, so as to increase their overall fitness. Among spiders, sperm depletion and monogyny are known to be optimal male mating decisions either under strong sperm competition or as terminal investment strategies, when future mating opportunities are limited. In a medium-sized wolf spider, *Hogna radiata* (Latreille 1817), we investigated sperm depletion, terminal sperm investment and the potential for sperm competition in laboratory mating trials in which we allowed males and females to pair sequentially with two mates. Males mated with as many females as they encountered. We found no evidence of sperm depletion or terminal sperm investment, as mating duration and female fitness were unaffected by male mating history or age. Polyandry was rare and did not involve any clear fitness benefit to females, whereas pre-mating sexual cannibalism was a rather common outcome of mating interactions involving inseminated females. Our results indicate that *H. radiata* males are not sperm limited and support the hypothesis that the potential for sperm competition shapes the evolution of sperm allocation in this species. Monandrous females do not incur any fitness cost and could potentially benefit from cannibalizing their prospective mates.

Keywords: Lycosidae, mating systems, monandry, polygyny, sperm competition

Given males' reduced parental investment, sexual seleetion theory posits that males will generally maximize their fitness by mating with as many females as possible (i.e., polygyny: Trivers 1972). Since sperm allocation to a single female would likely reduce male fitness, theory predicts that monogyny will only evolve when males cannot monopolize multiple mates and/or paternal care is necessary (Emlen & Oring 1977). Among spiders, a taxon in which paternal care has never been observed, monogyny is a surprising common mating strategy (Knoflach & Van Harten 2000; Foellmer & Fairbairn 2003; Fromhage et al. 2007). For example, when mating with virgin females, *Nephila clavipes* males deplete their sperm in a single mating and thus cannot mate with a second partner (Christenson 1989; Rittschof 2011). Spider sperm depletion is usually accompanied by other male strategies like genital mutilation or sexual cannibalism (Michalik & Rittsehof 2011). For example, *Nephila fenestrata* and *Argiope aurantia* males break off their genital organs during insemination (Fromhage & Schneider 2006; Foellmer 2008), whereas *Latrodectus hasselti* and *L. geometricus* males may sacrifice themselves during copulation (Andrade & Banta 2002; Andrade 2003; Segoli et al. 2008).

Spider monogyny and sperm depletion might be an optimal male mating strategy, at least under particular conditions (Rittschof et al. 2012). Firstly, ejaculate costs may not be trivial, limiting sperm production (Dewsbury 1982; Michalik & Rittschof 2011). For example, *Nephila clavipes* males do not produce sperm during adulthood (Michalik & Rittschof 2011), and female *Pardosa astrigera* paired with previously mated males are more likely to fail to produce a clutch (Jiao et al. 2011a). Secondly, sperm competition may also promote monogyny (Fromhage et al. 2005; Fromhage et al. 2008). For example, in the false garden mantid *Pseudomantis albofimbriata*, sperm transfer during mating increases when males experience higher risks of sperm competition, as a strategy to outcompete rivals' sperm (Allen et al. 2011). Likewise, breaking off male genital organs and sexual cannibalism may limit female insemination by future prospective mates (Fromhage et al. 2005, 2007, 2008; Fromhage & Schneider 2006). Finally, male-biased mortality may contribute to the reduction of expected value of future mating to males, favoring sperm allocation to the current mate and monogyny (i.e., terminal investment hypothesis) (Andrade & Banta 2002; Segoli et al. 2006).

In this paper we analyze sperm allocation and mating strategies in a wolf spider, *Hogna radiata* (Latreille 1817). We conducted two experiments in the laboratory, using mating duration and female fitness as indirect measures of sperm alloeation. The first experiment assessed sperm depletion and sperm terminal investment. In this experiment we allowed males of variable ages to pair sequentially with two unmated females and measured the effect of male mating history and age on mating duration and female fitness. To control for mate encounter rate prior to the experiment we used previously isolated unmated males (simulating a reduced mate encounter rate). We predicted that if males deplete their sperm in a single mating (sperm depletion hypothesis), mated males would mate for less time and/or females mated only to these males would have lower fitness (1). If males show terminal sperm investment, mating duration and reproductive output (measured as female fitness) will increase with increasing male age (2).

The second experiment tested the potential for sperm competition. Because females must mate with more than one partner for sperm competition to occur, we paired females sequentially with two unmated males. If sperm competition is a normal part of the reproduetive biology of this species, we predicted that females would usually accept multiple mates and polyandry would relate positively to female fitness (3). Alternative to sperm competition, females may mate multiply to avoid sperm limitation, which might involve an increased likelihood of remating by females experiencing reduced sperm supply (i.e., shorter first mating) (4).

METHODS

The species.—*Hogna radiata* (Latreille 1817) is a medium-sized European wandering wolf spider. It is very common and widely distributed in the Iberian Peninsula, where it inhabits a variety of terrestrial habitats, from wet to relatively arid sites, dominated by grassy vegetation. In the laboratory, they have a rather short post-embryonic development of ca. 10 months and 10–12 molts (C. Fernández-Montraveta unpubl. data). In central Spain, males and females mature early in summer and mating occurs shortly afterwards. The moderately long-lasting mating includes repeated and alternated insertions of both male emboli into the female genital tract. Females produce an egg sae within a few weeks following mating, while males disappear completely from natural populations only a few weeks following maturation. As in other wolf spiders so far studied, females carry egg sacs and then spiderlings until dispersal. The species is semelparous, but females may produce more than one egg sac during their only reproductive season (C. Fernández-Montraveta unpubl. data). Sexual size dimorphism is only moderately female-biased; females are on average 10% larger than males (C. Fernández-Montraveta unpubl. data).

General procedures.—Male and female *H. radiata* mating history cannot be properly assessed morphologically, as there are no external signs of fertilization. We therefore investigated male sperm depletion, terminal investment and the potential for sperm eompetition using laboratory-matured spiders in laboratory mating trials. For experiments, we captured subadults (immature but sexually differentiated spiders cf. Foelix 1996) late in spring near Madrid (central Spain). We used headlamps to capture spiders by hand during the night, which facilitates capture without physical damage. In the laboratory, we housed spiders individually in 1-l plastic containers, keeping the spiders visually isolated. We provided sand as substrate, on top of which we placed a small leaf as refuge. We kept laboratory conditions in a natural light regime provided by artificial lighting and 25 ± 2°C ambient temperature. To ensure humidity, we sprayed water on each spider container twice a week. We reared spiders on a monotypic diet (blowflies) provided three times a week.

We checked molting of eaeh spider on a daily basis until maturation, recognized as eomplete development of female and male external genitalia. We calculated spider age as the number of days after date of maturation. For each spider, we measured the maturation size as the maximum prosoma width to the nearest 0.05 mm (SZX9 Olympus mieroscope with 57x magnification provided with a mierometer). Additionally, we weighed spiders to the nearest 0.1 mg (Mettler Toledo ABS54 electronic balance) before each mating trial and again before oviposition. We kept spiders at the laboratory until their natural death. Voucher specimens of spiders used in this study are deposited at Museo Nacional de Ciencias Naturales – CSIC (Madrid, Spain).

Mating trials.—As an experimental arena we used a plastic cylinder (42–45 cm in diameter, transparent walls) fixed to a wooden platform. The cylinder was filled half-full with sand. A new clean filter paper placed on top of the sand prevented any chemical contamination of the surface, and a couple of small pieces of cardboard (5 × 5 cm) on top of the paper and close to the terrarium wall provided refuge to spiders. In preparation for a mating trial, we introduced a mature female *H. radiata* into the experimental arena and left her there for 24 h, allowing her to lay draglines. Wolf spider female draglines are known to contain silk-bound sex-pheromones that elicit a remarkably strong courtship response by conspecific males (Tietjen 1977; Fernández Montraveta & Ruano Bellido 2000). After checking visually for the presence of draglines, we gently placed a randomly chosen male ca. 10 cm from the female. We videotaped male and female behaviors (JVC TK-C621 video camera, JVC SVHS HR-S7000 video recorder, FOR-A VTG 55 video timer) for a maximum of 30 min if courtship and/or mating did not oceur, otherwise until mating was over. Following mating, we checked females daily, noting the day of egg sac production and spiderling hatching, the egg sac weight, and the number of spiderlings hatching from the egg sac.

Experimental design.—We conducted two independent mating experiments. Experiment 1 used unmated females to test for sperm depletion (1) and terminal investment (2). Specifically, we tested whether, compared to unmated males, mated males mated for less time and/or had lower fitness (Prediction 1) and whether older males mated for longer and experienced an increased reproductive output, measured as female fitness (Prediction 2). To manipulate male mating history, we tested each male twice, first as unmated and again several days following mating, paired with different females. Male age varied randomly. Because female mating history may affect the female response to courting males, we only used unmated females in this experiment. To control for the likely effect of hunger on the female response to courting males, and particularly on sexual cannibalism (Wilder & Rypstra 2008), we always provided females with food 24 h before each mating trial. Female age may also affect the female response to courting males (Wilgers & Hebets 2012), but the delay between consecutive trials prevented controlling for female age. In this experiment we used 21 spiders (7 males and 14 females).

In Experiment 2, designed to test the potential for sperm competition, we tested predictions 3 and 4. Particularly, we measured whether polyandry was a common output of mating interactions and related positively to female fitness (Prediction 3) and whether the likelihood of female remating increased following relatively short first matings (Prediction 4). We allowed each female to mate twice. In this experiment we controlled female age. We only used 16 to 18 day–old virgin females for the first mating and re-mated females 2 d afterward. We also controlled for male and female hunger by providing spiders with food 24 h before being used in a trial and presented a single unmated male to every female per trial. In this experiment we used 39 spiders (13 females and 26 males) in all.

For mating trials, we measured the interaction outcome (courtship, mating, or sexual cannibalism) and the courtship and mating durations. Also, we estimated fitness from 1) female mass gain from mating to oviposition, 2) egg sac production, 3) number of egg sacs produced, 4) egg sac weight, 5) time to egg sac production (days elapsed since mating until the production of the first egg sac) and 6) hatching success. Lastly, we calculated (7) the developmental time inside the egg sac as the number of days from egg sac production to spiderling hatching, and (8) the number of hatched spiderlings.

Table 1.—Results of Experiment 1, summarizing male and female body mass and size and female mass gain from mating to oviposition, courtship and mating duration and several estimates of female fitness (mean ± SE), depending on the male mating condition (mated, unmated). Results of statistical tests, including *P* values. Significant differences in bold.

Trait	Male condition		Test statistic	
	Unmated	Mated	Value	*P*
N	7	7		
Female body size (mm)	6.7 ± 0.4	7.2 ± 0.03	$X^2_1 = 1.9$	0.2
Female mass gain (g)	0.22 ± 0.03	0.32 ± 0.03	$X^2_1 = 4.6$	**0.03**
Male body mass (g)	0.3 ± 0.04	0.3 ± 0.03	$F_{1,6} = 0.1$	0.9
Male age (days)	15 ± 4	30.7 ± 3		
Courtship duration (min)	12.6 ± 4.2	8.8 ± 1.9	$F_{1,6} = 0.8$	0.4
Interaction outcome (%)				
Mating	100	100		n.s.
Sexual cannibalism	0	28.6(2)	Fisher exact	0.2
Mating duration (min)	31.2 ± 2.2	26.6 ± 6.1	Wilcoxon signed	0.6
Number of egg sacs (%)				
1	71.4(5)	57.1(4)	Fisher exact	0.5
2	28.6(2)	42.9(3)		
Time to egg sac production (days)	24.3 ± 1.1	16.4 ± 1.5	$F_{1,12} = 17.1$	**0.01**
Egg sac weight (g)	0.21 ± 0.03	0.28 ± 0.03	$F_{1,12} = 2.3$	0.1
Hatching success (%)	71.4(5)	71.4(5)	Fisher exact	0.7
Time to cocoon hatching (days)	29.8 ± 0.5	30.8 ± 1.3	$F_{1,8} = 0.5$	0.5
Spiderling number	115.8 ± 15	155.4 ± 54.6	$X^2_1 = 0.6$	0.4

Statistical analyses.—In Experiment 1, we used mating duration and female fitness as estimates of sperm allocation, and analyzed whether any of these parameters varied, depending on the male mating history and age. In Experiment 2, we measured multiple mating by females, its fitness eonsequenees and its relationship with first mating duration as a proxy measure of sperm supply. We present quantitative data as mean ± 1 SE. We used parametric statistics (General Linear Models) whenever possible. We inspected data visually for outliers, which we excluded from final analyses. We tested for normality (Shapiro-Wilks test), homoscedasticity (Levene test) and model fitting. When data were not homoscedastic we used Generalized Linear Models (Normal Identity link function). We used the Log likelihood ratio, compared to the Chi-square test, to check for statistical significance. When data failed to fit normality we applied non-parametric statistical tests. We used the IBM SPSS 19.0 package (IBM Corp.) for statistical tests.

RESULTS

Sperm depletion and terminal investment.—In Experiment 1, unmated male age ranged from 6 to 32 d, and time between the two male mating trials was 15.7 ± 1.9 d, representing a long time span. However, males did not experience any significant body mass loss between consecutive mating trials, and females tested with unmated and mated males were also similar in size (Table 1). All experimental females laid draglines on the terrarium surface, and males always courted upon contact with female draglines. Courtship duration did not differ depending on the male mating history, and courtship interactions always led to mating (Table 1). Male mating history did not affect mating duration (Table 1). We observed a few instances of post-mating sexual cannibalism, always in mating trials involving mated males, but the relationship between male mating history and sexual cannibalism was not significant (Table 1).

As expected, females increased their body mass before oviposition, and females paired with mated males gained relatively more mass (Table 1). All females paired in mating trials succeeded in producing an egg sac, and a few females produced two egg sacs. Male mating history did not affect the number of egg sacs produced by females, but females paired with mated males took less time to produce their first egg sac (Table 1). Male sperm allocation was unaffected by male mating history in all measures of female fitness (egg sac weight, developmental time, hatching success and female fecundity: Table 1).

Male age was unrelated to mating duration, both for unmated (Spearman correlation test: $\rho = -0.2$, $P = 0.3$) and for mated males ($\rho = 0.4$, $P = 0.2$). Likewise, there was no significant relationship between male age and female mass gain [Spearman correlation tests: $\rho = 0$, $P = 0.5$ (unmated); $\rho = -0.3$, $P = 0.3$ (mated)] or fecundity [Spearman correlation tests: $\rho = -0.6$, $P = 0.3$ (unmated); $\rho = -0.6$, $P = 0.3$ (mated)], whichever the male mating history.

Sperm competition.—In Experiment 2, males started courtship immediately upon contacting a females' draglines with forelegs or pedipalps. Courtship was significantly shorter in trials involving unmated females (2.9 ± 0.13 min), compared to mated females (12.4 ± 1.7 min, Wilcoxon signed test: $P = 0.02$). Likewise, female mating history affected the female response to male courtship, and polyandry was extremely rare. Courtship interactions involving unmated females always led to mating, compared to only 15.4% of the interactions involving mated females (McNemar test: $P = 0.001$, $n = 13$). Unmated females never attacked males prior to mating, compared to 84.6% ($n = 13$) of the inseminated females. Nearly half of these females cannibalized their prospective

Table 2.—Results of Experiment 2 summarizing (mean ± 1 SE) morphological data (male and female body size and mass), mating duration and several estimates of female fitness depending on the female mating strategy (monandrous or polyandrous). Results of statistical tests, including the *P* values.

Trait	Reproductive status		Test statistic	
	Polyandrous	Monandrous	Value	*P*
N	2	11		
Female body size (mm)	6.8 ± 0.8	6 ± 0.2	$F_{1,11} = 1.3$	0.5
Female body mass (g) maturation	0.7 ± 0.2	0.54 ± 0.06	$F_{1,11} = 0.9$	0.4
Female body mass (g) mating	0.73 ± 0.2	0.64 ± 0.5	$F_{1,11} = 0.4$	0.5
Male 1 body size (mm)	5 ± 0.2	5.1 ± 0.2		
Male 2 body size (mm)	5.1 ± 0.02	5.5 ± 0.2	$F_{1,9} = 0.8$	0.4
Male 1 body mass (g) mating	0.3 ± 0.02	0.3 ± 0.03		
Male 2 body mass (g) mating	0.3 ± 0.03	0.4 ± 0.04	$F_{1,11} = 2.7$	0.1
First mating duration (min)	15.7 ± 2.7	33.3 ± 8.1	Mann-Whitney	0.2
Cocoon production (%)	50(1)	90.1(10)	Fisher exact	0.3
Cocoon number (%)				
1	0	90(9)	Fisher exact	0.2
2	100(1)	10(1)		
Female body weight (g) oviposition	0.64	0.8 ± 0.06		
Female mass gain (g)	0.09	0.10 ± 0.03		
Time to cocoon production (days)	16	16.1 ± 2		
Cocoon weight (g)	0.16	0.2 ± 0.02		
Time to cocoon hatching (days)	35	31.3 ± 0.6		
Spiderling number	35	89.8 ± 19.8		

mates (5 out of 11, or 45.4%). A few (15.4%, $n = 13$) unmated females attacked males following mating, but these attacks never led to sexual cannibalism.

We found no morphological difference between monandrous and polyandrous females, nor between males succeeding or failing to mate with inseminated females (Table 2). Polyandry had no effect on female reproductive output (egg sac production, number of egg sacs produced, or time to first egg sac production: Table 2). Spiderlings hatched from all first egg saes produced by females. Beeause only one polyandrous female sueceed in reproducing, we could not test further for effects of mating history on female fitness. However, for this female, fitness estimates were within the range of monandrous females, except for spiderling number, which was lower (Table 2). Finally, first mating duration did not differ significantly between monandrous and polyandrous females (Table 2).

DISCUSSION

Our experimental results indicate that *H. radiata* males do not deplete their sperm in a single mating, but are capable of mating with more than one female. We found no support for the terminal investment hypothesis, as males in our first experiment effectively inseminated both females they encountered, in spite of increasing age and prior mating experience. Males who mated more than once therefore had an advantage, and we conclude that this species shows a potentially polygynous mating system. We found no evidence for sperm competition, as polyandry was rare and multiple mating did not entail any fitness benefit to females in our laboratory mating trials.

Supporting the hypothesis that males are not sperm limited and allocate sperm similarly among successive mates, we failed to find any effect of male mating history on offspring number. Moreover, and contrary to the sperm depletion hypothesis, females mating with mated males took less time to produce an egg sac and gained more body mass before oviposition than those mating with unmated males. We interpret the increased mass gain as indicating that mated males had supplied resources other than sperm during mating, particularly nutritional resources. In this experiment, some females mating with mated males cannibalized their partners, and sexual cannibalism is known to have positive effects on female fecundity in a closely related wolf spider (Rabaneda-Bueno et al. 2008). Nutritional resources supplied by cannibalized males would explain the average increased mass gain experienced by females paired with mated males.

In this experiment, the reduced time to oviposition experienced by females paired with mated males did not necessarily result in a fitness benefit. Second mating by males also occurred extremely late in the reproductive season, and reducing the time to oviposition actually compensated delayed mating and ensured no delayed hatching. Females mating later seem to invest their resources to produce spiderlings earlier, thus limiting any negative effect of hatching time on the likelihood of spiderling survival, which could indicate that female reproduction is time-limited.

We found no support for the terminal investment hypothesis, as there was no evidence that sperm allocation by males depended on the value of future mating. In central Spain, the *H. radiata* mating season ranges from mid-July to early August (C. Fernández-Montraveta, unpubl. data), and the age at which we tested males in laboratory mating trials (up to 43 d) greatly exceeded the time at which males survive under natural conditions. Timing of reproduction is known to negatively affect spider reproductive success (Rittschof et al. 2012), and the value of future mating is expected to decrease as the males get older. Moreover, we kept spiders isolated except for mating trials, and males would have experienced an extremely low mate encounter rate (roughly a single female every two weeks). If terminal investment shapes male mating strategy, both getting older and experiencing a reduced mate

encounter rate should have affected sperm allocation, with males increasing the sperm allocated to their current partner the older they get. No such trend appeared in our experiments.

Besides terminal investment, spider monogyny has been also related to sperm competition (Fromhage et al. 2008), with reduced potential for sperm competition in polygynous species. As predicted under this hypothesis, we found no evidence for sperm competition in *H. radiata*. The only polyandrous female reproducing in our experiment produced two egg sacs, but spiderlings did not hatch from the second egg sac. Moreover, the number of spiderlings produced by this female was actually not above, but below the range of values representing the reproductive output of monandrous females. Again, a high percentage (roughly 50%) of monandrous females in Experiment 2 cannibalized their second prospective mate, and sexual cannibalism could improve female reproductive output more than polyandry. The likely relationship between *H. raditata* sexual cannibalism and female fitness requires further testing.

If sexual cannibalism has a positive effect on female fitness, and a single mating supplies sperm enough to fertilize female eggs, then mating with a single male and cannibalizing all prospective mates would be the optimal reproductive strategy from the female point of view. This interpretation would indicate that *H. radiata* monandry is female-driven, as has been recently demonstrated in a closely related wolf spider (Jiao et al. 2011b). In our experiment, most females followed this strategy, and only a tiny portion mated twice. In species in which polyandry is female-driven, multiple mating by females has been related either to sperm competition or to sperm supply, which is demonstrated by a negative relationship between multiple mating and first mating duration (Weldingh et al. 2011). We found no relationship between number of matings and mating duration in *H. radiata*, though first mating duration by polyandrous females represented, on average, roughly half the mean mating duration by monandrous females. Again, lack of significant differences might relate to our small sample size, and we cannot completely discard the hypothesis that *H. radiata* multiple mating relates to lack of sperm supply.

In our experiments we used three indirect estimates of sperm allocation, based on male behavior (i.e., courtship duration, mating occurrence and mating duration), but did not measure sperm transfer directly. The relationship between spider mating duration and sperm transfer is complex (Schneider et al. 2005; Wilder and Rypstra 2007; Herberstein et al. 2011). For example, higher mating duration might not relate to effective insemination, but to reproductive failure (cf. Jiao et al. 2011a). However, we also included direct estimates of female fitness that pointed in the same direction. We therefore conclude that *H. radiata* shows a female-driven monandrous mating system. The risk of potential sperm competition seems to be low, which favors a polygynous mating system. Sexual cannibalism probably improves female fitness more than polyandry, which could relate to sperm supply. These possibilities need further investigation.

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Courtship behavior in European species of the genus *Pardosa* (Araneae, Lycosidae)

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Abstract. The study of courtship behavior provides a useful tool for identifying cryptic species due to the qualitative differences that can be observed in closely related species. Here, we present an overview of visual courtship displays of 26 European species of the genus *Pardosa* C.L. Koch 1847, including new quantitative and qualitative data. Thirty-five recurring courtship elements are described and illustrated by means of drawings, photos and videos (available online). In general terms, complex movements of the palps, the first pair of legs and the abdomen characterize courtship displays in the genus *Pardosa*. The most complex movements are performed by the palps, while legs and abdomen mainly oscillate in the air or vibrate on the substrate. We noticed a high level of complexity in almost all of the species, both in terms of sensory modes involved and number of courtship elements composing the displays. No apparent relationships emerged when considering ethological similarities among species, attesting to the relative independence between morphological and ethological characters.

Keywords: Displays, sexual communication, visual signaling behavior, wolf spiders

The lycosid genus *Pardosa* C.L. Koch 1847 is one of the largest spider genera in the world; Platnick (2012) lists more than 500 species, with about 60 valid species occurring in Europe. *Pardosa* spiders are diurnal wandering predators occurring in a variety of environments, but predominantly in open habitats. Several species have been placed in phenetic species groups based on similarities in the copulatory organs (e.g., Zyuzin 1979; Almquist 2005). Morphological characters, especially in females may overlap between species (Den Hollander & Dijkstra 1974), and it can be difficult to find distinct criteria for species identification (Vlijm & Dijkstra 1966; Töpfer-Hofmann et al. 2000). On the other hand, courtship displays, which are characterized by redundant and stereotyped movements, are species-specific and are considered diagnostic for species identification (Den Hollander & Dijkstra 1974).

Courtship behavior plays an important role in the pre-mating isolation mechanism between species. Since species may occur in syntopy or, at least, can be found in neighboring habitats and/or in the same season, ecological or phenological isolation seems in many cases to be insignificant. Under these circumstances, courtship behavior, in association with semi-ochemicals, may be regarded as an important barrier to prevent hybridization among *Pardosa* species (Töpfer-Hofmann et al. 2000).

According to Land (1985) and Rovner (1996), wolf spiders possess good image resolution but lack color perception. The male usually performs visual courtship display using complex movements of palps, forelegs and abdomen (Stratton 1985). In addition, males of some species have epigamic characters like divergent hairiness, deviant pigmentation of the first pair of legs and palps or a combination of these features (Kronestedt 1979a; Stratton 1985; Mc Clintock & Uetz 1996; Scheffer et al. 1996; Hebets & Uetz 1999, 2000; Taylor et al. 2005; Framenau & Hebets 2007). Moreover, communication by percussion, stridulation, and vibration of body parts is also well documented in wolf spiders (Rovner 1967, 1975; Buckle 1972; Kronestedt 1973, 1990, 1996; Stratton & Uetz 1981, 1983), with regard to both substrate-borne and air-borne vibrations. Chemical communication generally involves contact or airborne sexual pheromones and mediates different kinds of interactions (Rovner 1968; Tietjen 1979a, b; Stratton & Uetz 1981; Tietjen & Rovner 1982; Stratton 1985; Kronestedt 1986; Uetz 2000; Barth 2002; Uetz & Roberts 2002; Roberts & Uetz 2004). Contact pheromones are associated with female draglines (Hedgekar & Dondale 1969) and are detected by chemosensitive hairs in the male (Foelix & Chu-Wang 1973; Kronestedt 1979b).

Despite the potential in using courtship displays for distinguishing closely related species of the genus *Pardosa*, literature about European species is rather scarce (Den Hollander & Dijkstra 1974; Töpfer-Hofmann et al. 2000; Kronestedt 2007; Chiarle & Isaia 2013). With regard to North American species of *Pardosa*, studies have focused on *Pardosa falcifera* F.O.P.-Cambridge 1902 and *P. zionis* Chamberlin & Ivie 1942 (Vogel 1970) and *P. dromaea* (Thorell 1878) and *P. groenlandica* Thorell 1872) (Dondale & Redner 1990; Dondale 1999). In Japan, Suwa (1980, 1984) and Tanaka & Suwa (1986) studied the ethological differences among closely related species in the *P. laura* Karsch 1879 complex.

In view of the lack of ethological research on the genus *Pardosa,* in this paper we hope to contribute to the body of knowledge on this genus of wolf spiders, emphasizing its potential as a good model for studies on sexual communication. More specifically, the aims of this study are 1) to gather available information on the courtship displays of the European species of the genus *Pardosa*, integrating them with new observations; 2) to provide a detailed list and a description of the courtship elements (sensu Lehner 1998)

Table 1.—Sequences of courtship elements (see text for abbreviations) and previous published descriptions considered in this work. Courtship elements are grouped in the same row when they occur simultaneously.

Species	Courtship Elements Sequence	Previous courtship description
P. amentata	P_SE + A_TW	Locket (1923), Bristowe & Locket (1926), Schmidt (1957),
	P_Q + L_Q + A_Q	Bristowe (1958), Vlijm et al. (1963), Vlijm & Dijkstra (1966), Vlijm et al. (1970), Cordes (1995)
P. bifasciata	P_W + L_WA	none
	B_H + L_WH + P_Q	
P. schenkeli	P_P	Kronestedt (2005)
	P_P + B_B + A_TA + L_WH	
P. lugubris	B_P	Vlček (1995: species A), Töpfer-Hofmann et al. (2000)
	P_TC + L_LS + A_TW	
P. saltans	L_LS + P_TC + A_TW	Bristowe (1929: sub *Lycosa lugubris*); Vlijm & Dijkstra (1966: sub *P. lugubris*), Vlček (1995: species B), Töpfer-Hofmann et al. (2000)
	L_P + P_SRL + A_TW	
	P_TRL	
	B_E	
P. agrestis	P_LWJ + L_O + A_TW	Kronestedt (1979a)
	A_TW + L_Q	
P. agricola	P_W + L_O + A_TW	Kronestedt (1979a)
P. blanda	P_Q + A_Q	none
	B_H + L_WH	
	P_UDJ + L_T + A_TA	
	A_TW + P_W	
P. mixta	B_SW	none
	L_R	
	P_ARJ + A_TW	
	L_WH + P_Q + A_TA	
P. monticola	A_TW	Kronestedt (1979a)
	P_RL	
P. palustris	B_SW + P_W + A_TW	Kronestedt (1979a)
P. purbeckensis	P_SWR + L_R	Bristowe (1929), Kronestedt (1979a)
	P_SWR + L_O + A_TW	
P. torrentum	A_TW	none
	A_TA	
	B_SW + L_O + P_UDJ	
P. nigra	P_C + L_R	none
	B_H + L_WH	
P. nigriceps	P_CC + L_Q + A_TW	Locket (1923), Bristowe & Locket (1926), Bristowe (1958), Vlijm & Dijkstra (1966)
P. hortensis	P_SC + L_Q + A_TW	Vlijm & Dijkstra (1966)
P. proxima	L_R + P_W + A_TW	Den Hollander & Dijkstra (1974), Chiarle & Isaia 2013
	B_H + L_T + P_CR + A_TW	
	P_CR	
P. vlijmi	B_B	Den Hollander & Dijkstra (1974), Chiarle & Isaia 2013
	B_H + A_TW + P_CR + A_TA	
	P_CR	
P. pullata	B_J	Bristowe & Locket (1926); Vlijm & Borsje (1966); Hallander (1967); Den Hollander (1971); Den Hollander et al. (1973); Kronestedt (1979a, 2007)
P. pyrenaica	P_W + B_J	Kronestedt (2007)
P. fulvipes	P_RL + A_TW	Kronestedt (1979a)
	L_WA + A_TW	
P. prativaga	A_TA + P_CR	Den Hollander et al. (1973), Kronestedt (1979a)
	P_Q	
	A_TA + P_CR + P_Q	
	B_H + A_TA + P_CR	
	B_R	
	B_E	
P. riparia	B_P	Kronestedt (1979a)
	P_W + A_TW	
P. sphagnicola	A_TA + P_Q	Den Hollander et al. (1971: sub *P. prativaga fulvipes*, 1973), Kronestedt (1979a)
	B_H + A_TA + L_T + P_CR	
	B_R + A_TA	

Table 1.—Continued.

Species	Courtship Elements Sequence	Previous courtship description
P. wagleri	P_LJ + A_Q + A_TA B_P + L_WH + P_LJ + A_Q + A_TA P_RL	Chiarle et al. (2010)
P. saturatior	P_Q + A_Q + A_TA B_P + L_WH + P_Q + A_Q + A_TA P_RL	Chiarle et al. (2010)

recurring in *Pardosa*'s displays, with a goal of categorizing behavioral diversity in this genus; 3) to describe unknown courtship behaviors and add supplementary details to previous descriptions and 4) to compare different behaviors by focusing on similarities and differences among species.

FIELD COLLECTION AND MAINTENANCE

At the beginning of each paragraph describing courtship behaviors, we provide information on the material and number of observed courting males. We (AC, MI) collected the spiders in the field as adults or sub-adults and kept them in the laboratory, housed individually in cylindrical plastic containers (6 cm diameter, 2.5 cm high). Individuals were fed with two or three *Drosophila melanogaster* per week and provided access to a hydrated piece of cotton for water and humidity. We maintained spiders at 22 ± 1°C with a 10:14 h light:dark photoperiod. At the conclusion of each observation, spiders were preserved in ethanol for further molecular analysis. Voucher specimens are stored at the Museo Regionale di Scienze Naturali of Turin (Italy) (MRSN), at the Entomology Department of the Royal Belgian Institute of Natural Science in Brussels (Belgium) (RBINS) and at the Department of Life Sciences and Systems Biology, University of Turin (Italy) (DBIOS). Video samples of courtship display are stored at AC's personal website (www.ragnolupo.com) and are available on the Internet.

PUBLISHED DATA AND OTHER OBSERVATIONS

In an effort to provide a thorough history of the literature giving descriptions of courtship behavior of the 26 European species of *Pardosa*, we have included all of these speeies in our review. Most of the works were published between 1957 and 1979 (Table 1) and in only a few cases have these researchers presented and discussed quantitative data. In contrast, several recent descriptions are exhaustive and offer a comprehensive characterization of species' behaviors. In particular, this is the case with *Pardosa wagleri* (Hahn 1822) and *P. saturatior* Simon 1937 (Chiarle et al. 2010), *P. lugubris* (Walckenaer 1802), *P. saltans* Töpfer-Hofmann 2000, *P. pertinax* von Helversen 2000, *P. alacris* (C.L. Koch 1833) and *P. baehrorum* Kronestedt 1999 (Töpfer-Hofmann et al. 2000), *P. pullata* (Clerek 1757), *P. pyrenaica* Kronestedt 2007 (Kronestedt 2007), *P. proxima* (C.L. Koch 1847) and *P. vlijmi* Den Hollander & Dijkstra 1974 (Chiarle & Isaia, 2013).

Moreover, Kronestedt (1979a, 2005) published descriptions of the displays of *P. agricola* (Thorell 1856), *P. fulvipes* (Collett 1876), *P. monticola* (Clerck 1757), *P. palustris* (Linnaeus 1758), *P. agrestis purbeckensis* F.O.P.-Cambridge 1895, *P. riparia* (C.L. Koch 1833), *P. schenkeli* Lessert 1904, *P. sphagnicola* (Dahl 1908) and *P. prativaga* (L. Koch 1870) in local journals (in Swedish), for which we provide revised and improved descriptions gathered from the original super-8 films. In these cases, TK used a Beaulieu 4008 ZM super-8 camera provided with an Angénieux Macro-Zoom 1.9/8–64 mm lens. Spiders were collected as sub-adults or adults, kept individually in plastie containers with access to water and fed irregularly with *Drosophila melanogaster*. TK observed the spiders' behavior in a round plastic jar with the bottom (diameter 7 cm) covered with plastic foam. A segment of the jar was cut off and covered with a glass plate through which spiders were filmed and still-photographed using fiber optic illumination as a light source.

Regarding these observations, we provide the re-description of the basic courtship elements characterizing the average behavior of each species. When possible, we add quantitative data such as mean duration and standard deviation of courtship elements (given in seconds). Moreover, we provide sound recordings obtained by TK on *Pardosa schenkeli*, *P. prativaga* and *P. sphagnicola*. Sounds were recorded with a UHER 4200 tape reeorder from spiders performing courtship displays on a cardboard substrate in the observation jar. Data were analyzed using an audio program (Audacity).

Materials used in TK's studies are stored at the Swedish Museum of Natural History in Stockholm (Sweden) (NHRS).

NEW OBSERVATIONS AND RECORDING

Based on the observations of field-collected specimens, we provide new descriptions of the courtship behaviors of *P. bifasciata* (C.L. Koch 1834), *P. blanda* (C.L. Koch 1833), *P. mixta* (Kulczynski 1887), *P. nigra* (C.L. Koeh 1834) and *P. torrentum* Simon 1876. Moreover, on the basis of our new unpublished observations, we add new information about the courtship behavior of *P. agrestis* (Westring 1861), *P. amentata* (Clerck 1757), *P. hortensis* (Thorell 1872), *P. nigriceps* (Thorell 1856), *P. prativaga* (L. Koch 1870).

In order to obtain new descriptions or improve previous ones, AC and MI observed and recorded the male and the female in a glass arena (20 cm diameter, 5 cm high) on a sheet of absorbent paper to optimize the transmission of vibrations and the persistence of chemical traces (Rypstra et al. 2003; Chiarle et al. 2010). We used a white neon lamp as light source. We introduced the female into the arena for 15 min, in order to spread chemical traces and silk on the absorbent surface. We then placed the male in the arena and recorded its behavior for one hour, with two Canon HV30 cameras at 50i recording speed and then acquired with Adobe Premiere Pro CS3 (Adobe Systems Incorporated) at 1480×900 resolution. At the end of each trial, we cleaned the arena with paper and 75% ethanol in order to remove chemicals and silk cues. We provide the description of the courtships, including the basic

elements characterizing the behaviors, together with their frequency and duration (mean ± SD).

DESCRIPTION OF COURTSHIP ELEMENTS

Given the lack of comprehensive work on *Pardosa* courtship behavior, we provide a detailed list of the recurring courtship elements (*sensu* Lehner 1998) that we observed. Terminology is in most cases original, or inspired by previous work on *Schizocosa*.

For each part of the body involved in the behavior, we describe the basic courtship elements (CEs) recurring in the behaviors (Table 1); namely 17 elements for the palps, eight elements for the forelegs, three elements for the abdomen and seven elements for the whole body. Each CE is encoded by an alphabetical acronym starting with "P" if the behavior is related to palps, "L" for forelegs, "A" for the movement of the abdomen, and "B" in the case of general movement of the body. The second part of the code relates to the name of the CE (for example: P_SE = palpal semaphoring).

In order to help the reader with the visualization, the main CEs are illustrated in Figs. 1–16 for the palps, Figs.17–23 for the legs, Figs. 24–26 for the abdomen and Figs. 27–29 for the general body movements. Courtship elements are listed and described in the next section (in order of appearance within each category).

1) Palps

P_SE, *semaphoring* (Fig. 1): the male raises one palp above the eyes, then moves it forward and sideways with a quick movement of the trochanter-femur and patella-tibia joints. The cymbium is stretched upward at an angle of about 45° to the substrate. The male then raises the other palp, keeping it under the line of the eyes and moves it forward and laterally. The tip of the cymbium points forward and downward. The palps are then carried back to the starting position and stretched out. The whole movement starts again with the opposite palp.

P_Q, *quivering* (Fig. 2): a continuous fast movement of the palps, which are moved up and down along the vertical axis, involving the trochanter-femur and the patella-tibia joints, with the tip of the cymbium pointing downward.

P_W, *waving* (Fig. 3): the male slowly raises the palps and lowers them alternately with a vertical movement, involving the trochanter-femur and the patella-tibia joints. The cymbium points downward and at the end of the rotation is brought parallel to the substrate below the body. A slight tremble occurs during the whole process of waving.

P_P, *pointing* (Fig. 4): the palps are bent, forming a right angle between the tibia and femur. Then the male quickly stretches them in synchrony at the patella-tibia joint and maintains them parallel to the substrate, bringing them back quickly to the starting position, tips pointing down. The movement is repeated several times. Both palps may also be raised higher, involving the trochanter-femoral junction, but they never reach the height of the cephalothorax.

P_TC, *tip cycling* (Fig. 5): from a rather high posture, the male moves the palps circularly, from the left to the right, tips pointing outwards.

P_SRL, *slow raising-lowering* (Fig. 6): from a rather high posture, the male raises the palps and stretches them out alternately, with slow movements involving the patella-tibia junction.

P_TRL, *two-steps raising-lowering* (Fig. 7): raising of the palps in two-steps: at first the male raises the palps to the eye line (cymbium and tibia at right angle), then stretches them up. After the upright motion, the males lower the palps in synchrony.

P_LWJ, *lateral waving jerk* (Fig. 8): the male lifts one of the two palps with rapid jerky movements involving the patella-tibia junction. Within each movement, the cymbium points upward and forward. When the tibia is stretched up over the eyes, and the palp reaches maximum height, the male lowers the palp down rapidly. The cymbium points forward and then downward. At the end of the lowering process, movement is transferred to the other palp, which starts the in the same way. The palp that is not lifted is kept stationary or moved at the patella-tibia junction, in synchrony with the other palp. In some cases, the male lifts the two palps together.

P_UDJ, *up-and-down jerk* (Fig. 9): the male raises and lowers the palps along the vertical axis, involving primarily the trochanter-femur joints, bearing the cymbium slightly toward the body. During this movement, the palps are maintained in a slightly oblique position with the tip touching the substrate.

P_ARJ, *alternate raising jerk* (Fig. 10): the male lifts the palps alternately, in two or three steps (the movement can also be performed by one single palp). First they are stretched forward, oblique and kept in line with the eyes, involving the trochanter-femur and patella-tibia joints, then raised alternately over the cephalothorax, involving the trochanter-femur joints.

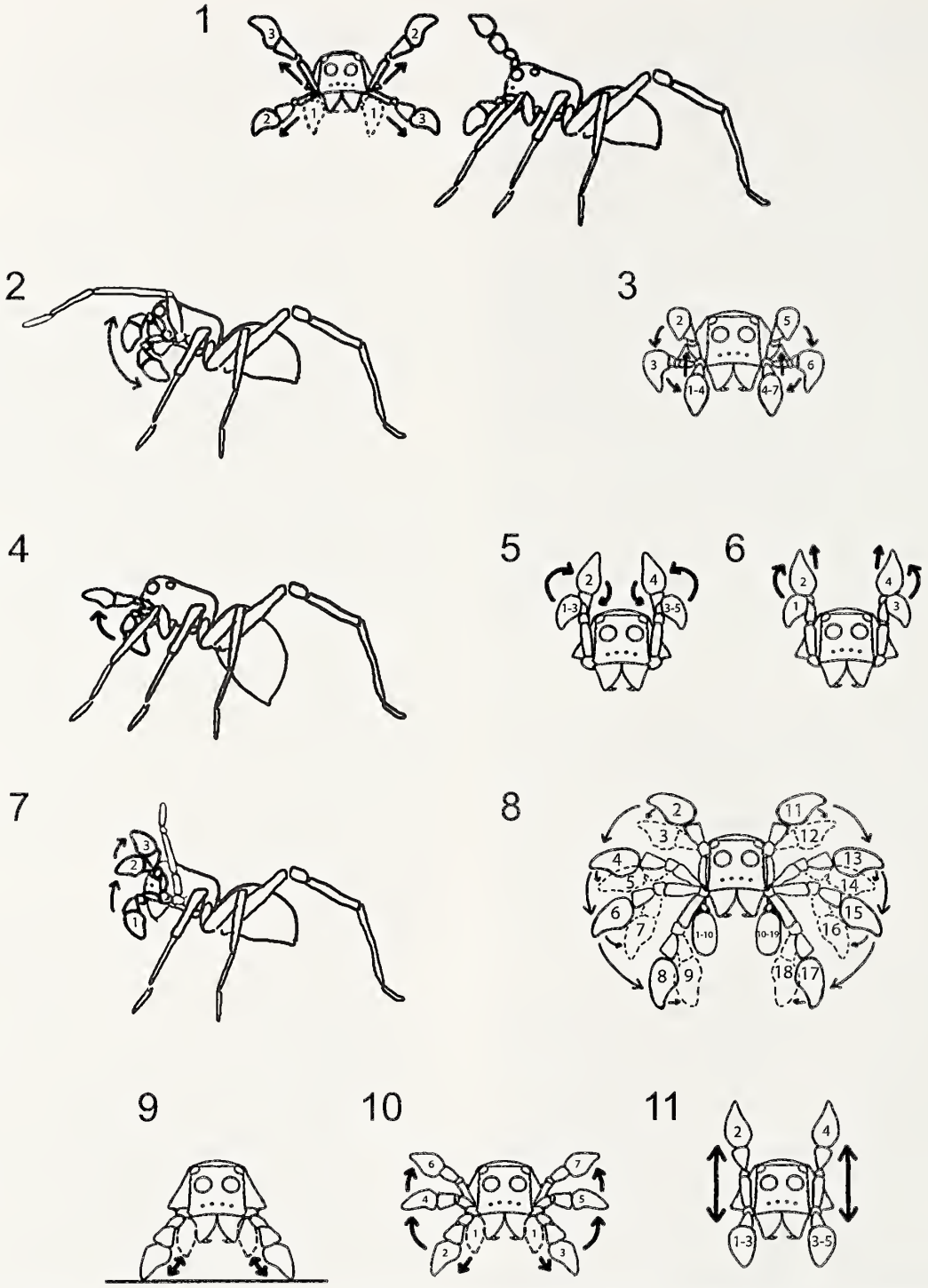
P_RL, *raising-lowering* (Fig. 11): the male raises one palp and quickly stretches it upward and forward, involving the trochanter-femur and patella-tibia joints. Then he lowers it, and brings it rapidly back to the starting position. This movement is repeated by the other palp or, less frequently, by the same palp.

P_SWR, *step-wise raising* (Fig. 12): the male raises the palps together in two or three steps. At first the palps are stretched forward in line with the eyes, involving the trochanter-femur and patella-tibia joints, and then they are raised over the cephalothorax involving the trochanter-femur joint. The male then often keeps the palps in the raised position, directed obliquely upwards. After that, the male slowly lowers both palps along the vertical axis with their tips directed downwards.

P_C, *circling* (Fig. 13): the male draws an ellipse with the two palps. The palps form a right angle between femur and tibia and move almost simultaneously. More precisely, the male raises the palps and allows their position to widen outwards slightly, then lowers them inwardly until they almost touch each other. The cymbia pointing downwards. This movement can be repeated several times.

P_CC, *continuous cycling* (Fig. 14): same as P_SC but without steps (palps are moved continuously).

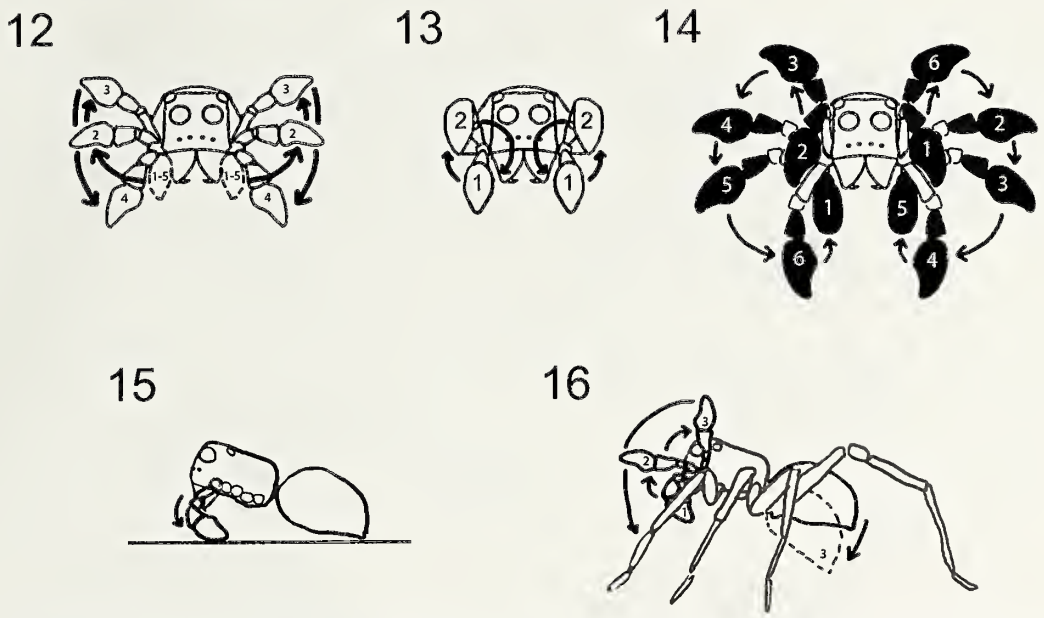
P_SC, *step cycling* (Fig. 14): the male raises one palp, tip pointing up (the movement involves trochanter-femur,



Figures 1–11.—Courtship elements in *Pardosa* species (palps). 1. Semaphoring (P_SE); 2. Quivering (P_Q); 3. Waving (P_W); 4. Pointing (P_P); 5. Tip cycling (P_TC); 6. Slow raising-lowering (P_SRL); 7. Two-steps raising-lowering (P_TRL); 8. Lateral waving jerk (P_LWJ); 9. Up and down jerk (P_UDJ); 10. Alternate raising jerk (P_ARJ); 11. Raising-lowering (P_RL). See text for abbreviations.

patella-tibia and tibia-tarsus joints). Then the same palp is lowered in 3–4 steps, performing lateral movements. During this movement, the tip of the palp draws half-circles until it returns to the resting position. This movement is repeated by both palps alternately or, in some cases, together. If one of the palps is not involved in the rotation, it remains still. During each step, the cymbium gently oscillates two or three times at the patella-tibia and tibia-tarsus joints.

P_CR, *cymbium rubbing* (Fig. 15): the palps are rubbed against the substrate with an active movement at the patella-tibia junction. The movement is mainly a consequence of the movement body (cymbium kept at ground level, rubbing on the substrate). Femur and tibia are kept at right angle.



Figures 12–16.—Courtship elements in *Pardosa* species (palps). 12. Step-wise raising jerk (P_SWR); 13. Circling (P_C); 14. Continuous\step cycling (P_CC, P_SC); 15. Cymbium rubbing (P_CR); 16. Low jerk (P_LJ). See text for abbreviations.

P_LJ, *low jerk* (Fig. 16): both palps are raised (bent at the coxa-femur and patella-tibia joints) and then lowered together, ending in a jerky, trembling movement.

2) Legs

L_WH, *whipping* (Fig. 17): the male raises the first pair of legs and stretches them until legs are almost perpendicular to the substrate. Immediately afterward, the legs violently hit the substrate or the female, if she is very close.

L_LS, *lateral stretching* (Fig. 18): the male stretches both forelegs laterally, upwards and outwards, in front of the female.

L_P, *pumping* (Fig. 18): the male stretches his legs in front of the female (L_LS) and then moves them slightly up and down. The movement involves the whole legs.

L_O, *oscillation* (Fig. 19): the male raises the first pair of legs, maintaining the femur almost perpendicular to the substrate (bent at the tibia junction). Tibia, metatarsus and tarsus are moved along the vertical axis with rapid small oscillations.

L_Q, *quivering* (Fig. 20): while moving toward the female, the male vibrates one or both front legs. The vibration may occur in contact with the substrate.

L_T, *tapping* (Fig. 21): the male raises the first pair of legs. Then, with a small vertical movement at the trochanter-femur junction, legs are lowered, hitting the substrate (tarsus kept approximately perpendicular).

L_WA, *waving* (Fig. 22): the male raises one foreleg at the trochanter-femur junction. During this movement, tibia, metatarsus and tarsus are stretched forward, parallel to the substrate. Subsequently, the foreleg is lowered until it touches the substrate. The movement can be repeated by the same foreleg or by the other one.

L_R, *raising* (Fig. 23): the male lifts the first pair of legs along the vertical axis, keeping the femur perpendicular to the substrate and the tibia perpendicular to the femur. Tibia, metatarsus and tarsus are stretched on the same line, parallel to the substrate. Forelegs can also be stretched with all segments perpendicular to the substrate.

3) Abdomen

A_TW, *twitching* (Fig. 24): a rapid vertical movement of the abdomen, which is kept parallel to the substrate, but not in contact with it. This CE can occur occasionally.

A_Q, *quivering* (Fig. 25): a series of fast movements of the abdomen, which is moved up and down along the vertical axis, parallel to the substrate, without touching it.

A_TA, *tapping* (Fig. 26): the abdomen is vigorously swung along the vertical axis, striking the substrate.

4) Body movement

B_SW, *sideways waving* (Fig. 27): the entire body moves slowly and rhythmically to the right and to the left. The waving involves the second, third and fourth pair of legs.

B_H, *hopping* (Figs. 28, 29): a series of generally jerky movements toward the female in the form of rapid jumps or short runs. The entire body often hits the substrate.

B_J, *jumping*: the male jumps onto the female in a single jump.

B_B, *bouncing*: the male quickly raises and lowers the whole body on the spot, with a series of small jumps.

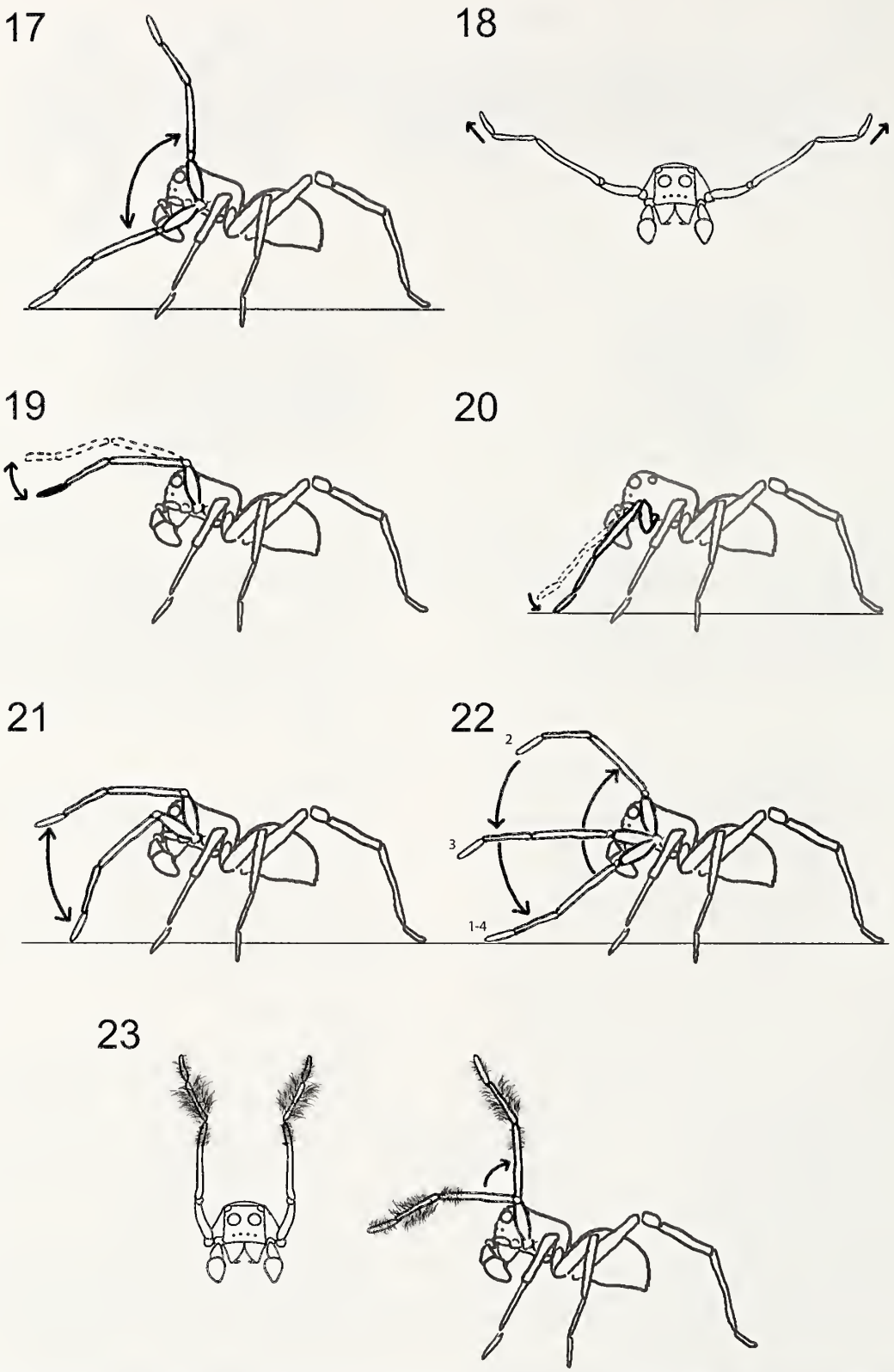
B_P, *pursuit*: the male pursues the female and gets closer. The chase ends when the female stops.

B_E, *encircling*: the male runs around the female, facing her and touching her legs.

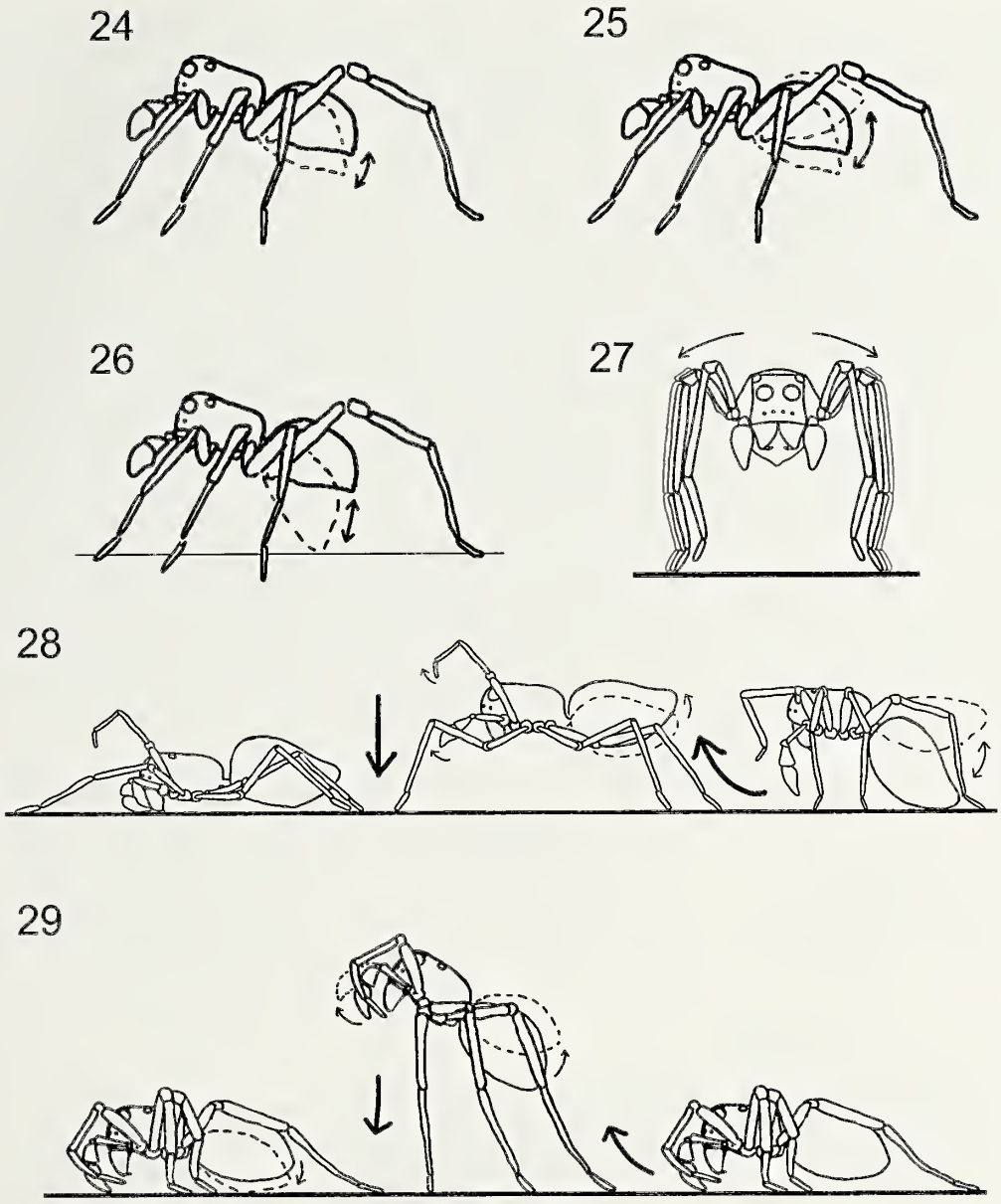
B_R, *retreat*: after contact with the female, a short backward run of the male, facing the female.

DESCRIPTION OF DISPLAYS

Species are listed according to Zyuzin's (1979) and Almquist's (2005) phenetic groups of species. For each group,



Figures 17–23.—Courtship elements in *Pardosa* species (forelegs). 17. Whipping (L_WH); 18. Lateral stretching/pumping (L_LS, L_P); 19. Oscillation (L_O); 20. Quivering (L_Q); 21. Tapping (L_T); 22. Waving (L_WA); 23. Raising (L_R, front and lateral views). See text for abbreviations.



Figures 24–29.—Courtship elements in *Pardosa* species (body and abdomen). 24. Twitching (A_TW); 25. Quivering (A_Q); 26. Tapping (A_TA); 27. Sideway waving (B_SW); 28. *P. prativaga* and *P. sphagnicola* hopping behavior (B_H); 29. *P. vlijmi* hopping behavior (B_H). See text for abbreviations.

we provide a brief introduction with taxonomical information concerning the species included in the group. For each species, we describe the average courtship display referring to the previous description of courtship elements. A list of materials examined (n = number of observed specimens) and a list of available literature on courtship are provided.

Pardosa amentata group

Only one European species is currently placed in the *P. amentata* group (Almquist 2005).

Pardosa amentata (Clerck 1757)

Material: ITALY: *Piedmont*, Province of Torino, Bobbio Pellice, Conca del Prà, alpine meadow (1732m a.s.l.), 9 November 2006, n = 5, A. Chiarle (MRSN); Province of Cuneo, Valdieri, alpine meadow (1763m a.s.l.), 4 April 2007, n = 6, A. Chiarle (MRSN). SWEDEN: *Uppland*, various localities in the vicinity of Stockholm, 1976–1982, n = 4, T. Kronestedt (NHRS).

Literature: Locket (1923), Bristowe & Locket (1926), Schmidt (1957), Bristowe (1958), Vlijm et al. (1963), Vlijm & Dijkstra (1966), Vlijm et al. (1970), Cordes (1995).

Description of the display: The male stretches out his palps with a fast movement (0.92 ± 0.18 sec, 101 observations, n = 5 individuals), one pointing obliquely upwards and forwards, the other obliquely downward and forward (P_SE). P_SE is then repeated after a few seconds (1.45 ± 0.41 sec, 49 observations, n = 5 individuals), reversing the position of the palps about three times (number of switching: 3 ± 1 times, 50 observations, n = 5 individuals). During P_SE the male performs A_TW, with an angle of about 45 °. Every time the palps switch their position, the male takes a step forward toward the female. While keeping the palps in this position, he

begins P_Q, together with L_Q and A_Q for about four seconds (4.03 ± 1.39 sec, 42 observations, n = 5 individuals), bringing them back to the starting position. Then the male repeats P_SE. The quivering of the first pair of legs results in a percussive signal, which was recorded by Cordes (1995).

Pardosa bifasciata group

The Palearctic *P. bifasciata* group is represented in Europe by two species: *P. bifasciata* and *P. schenkeli* (Zyuzin 1979).

Pardosa bifasciata (C.L. Koch 1834)

Material: BELGIUM: *Namur*, Viroinval, Nismes, sandy substrate (198m a.s.l.). 4 June 2009, n = 3, A. Chiarle (RBINS).

Literature: none.

Description of the display: The male alternates P_W and L_WA with slow movements touching the substrate. A series of about eight rapid stiff-legged hops toward the female (B_H, number of hops: 8 ± 4 times, 27 observations, n = 3 individuals; duration of a single hop: 0.23 ± 0.07 sec, 39 observations, n = 3 individuals) follows. At the same time, the male performs L_WH and P_Q.

Pardosa schenkeli Lessert 1904

Material: SWEDEN: *Dalarna*, Mora, pine forest on sand with *Cladonia* cover, patches with *Arctostaphylos uva-ursi* and bare sand, 26 May 1980, n = 2, T. Kronestedt (NHRS); Ore, Näset, similar habitat as previous, 23 May 1983, n = 1, T. Kronestedt (NHRS).

Literature: Kronestedt 2005.

Description of the display: The male starts with very rapid synchronous movements of the black palps with their tips pointing down (P_P). Rapid vertical bounces (B_B) of the body follow, and the palps continue to vibrate. During this behavior, performed on the spot, the distal part of the abdomen taps the substrate (A_TA) and performs rapid L_WH. The entire behavior is repeated after a short pause. Sounds from A_TA produced on substrate cardboard are illustrated in Fig. 51.

Pardosa lugubris group

The Palearctic species included in the *P. lugubris* group are morphologically almost identical, both in general appearance and genital organs. Furthermore, they can often be found in syntopy. The group was previously referred to as the *P. amentata* group (Zyuzin 1979), and only two European species were included in the group: *P. lugubris* and *P. amentata*. Töpfer-Hofmann et al. (2000) highlighted ethological differences within the group by comparing courtship behaviors and described two new species (*P. saltans* and *P. pertinax*). Moreover, they removed *P. amentata* from the *P. lugubris* group, both on behavioral and morphological bases. Kronestedt (1999) previously drew a similar conclusion on a morphological basis. At present, six species are listed for this group.

Courtship display in *Pardosa lugubris sensu lato* aroused the interest of several authors (Bristowe 1929; Vlijm & Dijkstra 1966; Hallander 1967; Vlček 1995; Töpfer-Hofmann et al. 2000). *Pardosa lugubris* shows a simple visual display: the male pursues the female (B_P) until she stops; and then he performs P_TC, L_LS, and A_TW while trying to mount her. In contrast, *Pardosa saltans* courtship behavior is rather complex and can be divided into several elements. Firstly, the male performs L_LS, P_TC, and A_TW and then starts to move the forelegs up and down (L_P) with low amplitude movements. At the same time, the palps are slowly raised alternately (P_SRL), and the abdomen twitches a few times (A_TW). After a few P_SRL, both palps are lowered slowly to the resting position. Immediately after, the male raises both palps (P_TRL) and runs in a circle around her (B_E). Given that only *P. lugubris* and *P. saltans* were filmed in the frame of this work, no other species belonging to this group were included in our analysis. In general terms, the other species belonging to this group (*P. alacris*, *P. baehrorum*, *P. caucasica* Ovtsharenko 1979, *P. pertinax*) perform slow motion palpal jerks and trembling in front of the female (for more details see Töpfer-Hofmann et al. 2000).

Pardosa monticola group

The *P. monticola* group is the largest group within the genus *Pardosa*, with 23 species occurring in Europe listed in Platnick's catalog (2012). Some of the species belonging to the *P. monticola* group are hardly distinguishable, especially the females. In contrast, males reveal some distinctive features allowing identification at the species level (Tongiorgi 1966b).

Several subspecies belonging to the *P. monticola* group have been described and eight of them [*P. agrestis purbeckensis* F.O.P.-Cambridge 1895, *P. agricola borussica* (Dahl 1908), *P. agricola fucicola* (Dahl 1908), *P. monticola ambigua* Simon 1937, *P. monticola minima* Simon 1876, *P. monticola pseudosaltuaria* Simon 1937, *P. palustris islandica* (Strand 1906), and *P. torrentum integra* Denis 1950] are considered valid (Platnick 2012). Most of the descriptions are generally based on phenetic characters, such as body and leg coloration.

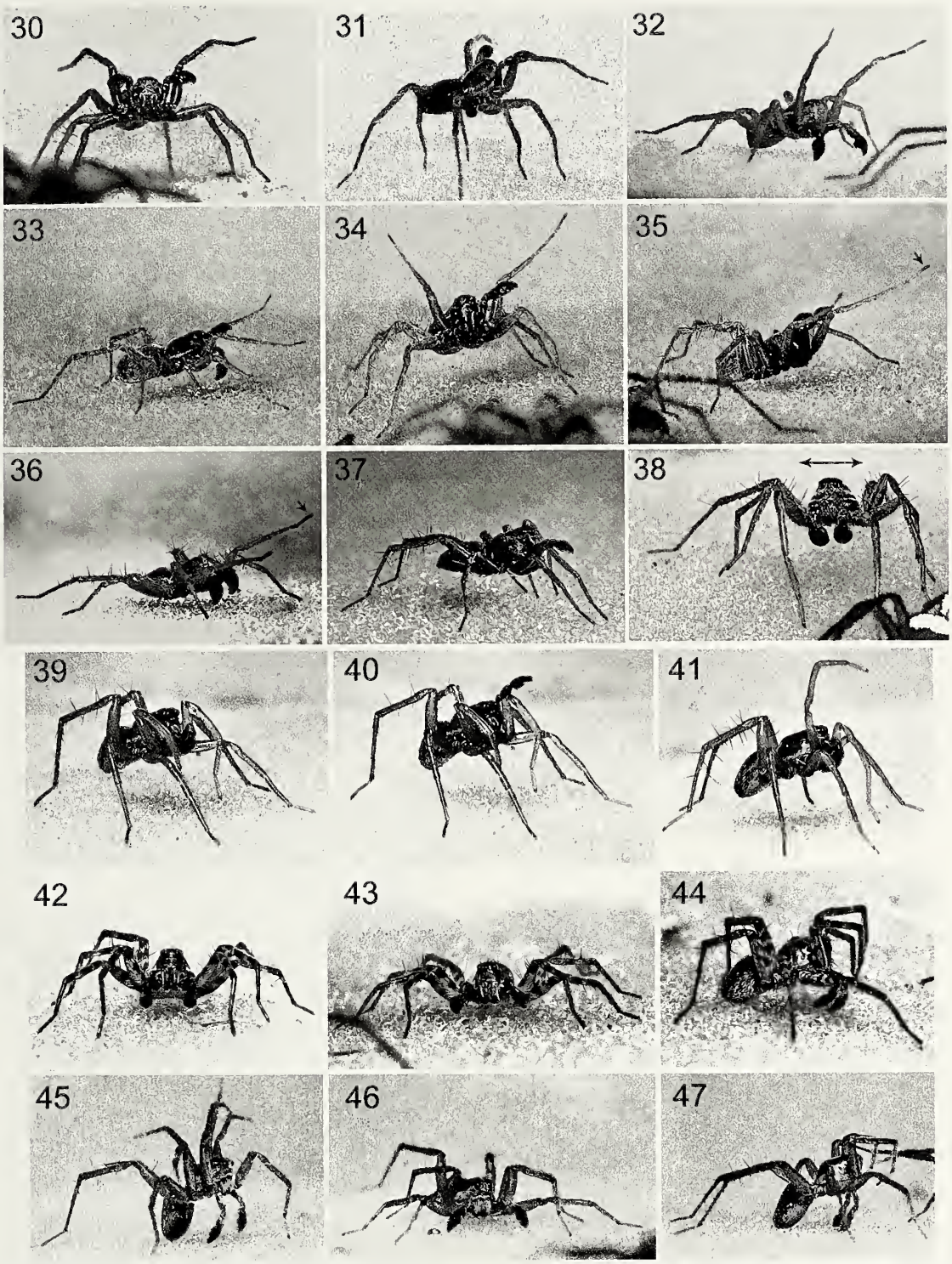
Among them, the taxonomic status of *P. agrestis purbeckensis* has been often debated. Currently, Platnick (2012) recognizes its validity in accordance with Alderweireldt & Maelfait (1993) and Pirchegger & Thaler (1999) who found no morphological differences in the male palp in order to support *P. purbeckensis* F.O.P-Cambridge 1895 as a valid species.

However, according to several authors (Knülle 1954; Tongiorgi 1966b; Heimer & Nentwig 1991), this hypothesis does not seem in accordance with other features such as differences in habitat preference (salt marshes and humid areas for *P. agrestis purbeckensis* versus meadows, arable lands and other open areas for *P. agrestis*) and morphological traits of the male forelegs. In addition, former descriptions of the courtship display (Bristowe 1929; Kronestedt 1979a) clearly support the elevation to species level.

Concerning the other species of this group, Kronestedt (1979a) described the courtship behavior of *P. agrestis*, *P. agricola*, *P. monticola* and *P. palustris* in a local Swedish journal. We provide the English description of the displays of these species and the first descriptions of the courtship behavior of *P. torrentum*, *P. blanda*, and *P. mixta* and provide further ethological evidence to support the elevation of *P. agrestis purbeckensis* to the species level.

Pardosa agrestis (Westring 1861)

Material: BELGIUM: *Brabant*, Tienen, sugar factory, sandy substrate (52m a.s.l.), 20 May 2009, n = 8, A. Chiarle,



Figures 30–47.—Male courtship postures in *Pardosa* species. 30–32. *P. purbeckensis*: 30–31. Palps and forelegs raised (P_SWR and L_R, frontal and posterior view); 32. Palps lowered and forelegs oscillating (L_O). 33–35. *P. agrestis*: 33–34. First legs moved slightly back and forth and palps jerkily raised in alternation or, sometimes, in synchrony (P_LWJ and L_O, lateral and frontal view); 35. L_O, arrow points at darkened tip of first tarsus; 36. *P. agricola*: L_O, arrow points at the mostly blackened tarsus. 37. *P. monticola*: palp raised (P_RL). 38. *P. palustris*: body slow sideways waving (B_SW). 39–41. *P. fulvipes*: 39. Initial posture with first legs raised; 40. Left palp raised (P_RL, cymbium pointing obliquely outwards); 41. Right first leg raised (L_WA, before straightened forwards and slowly lowered). 42–44. *P. prativaga*; 42. Initial position while performing few synchronous back and forth movements of the palps; 43. Rapid up and down movements of the obliquely outward directed palps (P_Q, tips directed downwards); 44. Close to female after a series of hops (B_H). 45–47. *P. sphagnicola*: 45. Elevated position during hopping behavior (B_H); 46. Low position during hopping behavior (B_H); 47. Close to female after a series of hops. (Photos T. Kronestedt). See text for abbreviations.

F. Hendrickx & J. Pétillon (RBINS). ITALY: *Piedmont*, province of Cuneo, Caraglio, Bottonasco, cultivated field (626m a.s.l.), 21 July 2007, $n = 5$, M. Isaia (MRSN). SWEDEN: *Uppland*, Stockholm, grassland, 1–3 June 1976, $n = 2$, T. Kronestedt (NHRS). Rådmansö, Gräddö, 2 June 1974, $n = 2$, T. Kronestedt (NHRS).

Literature: Kronestedt (1979a).

Description of the display: The male starts courting by alternately raising and lowering the palps while performing a series of about fourteen vertical movements of the cymbium (14 ± 3 times, 65 observations, $n = 5$ individuals; duration of one movement: 0.21 ± 0.02 sec, 36 observations, $n = 5$ individuals) drawing semi-circles with the palp (P_LWJ, Figs. 33–34). P_LWJ is performed by both palps alternately or, rarely, together. The forelegs are raised and moved slightly back and forth (L_O, Fig. 35), in synchrony with the movements of the palps (exposing the black tip of the tarsi) and the abdomen (AT_W). When the male stops performing P_LWJ, he steps toward the female (single step duration = 1.32 ± 0.30 sec, 36 observations, $n = 5$ individuals) and performs A_TW and L_Q. Then he starts P_LWJ, L_O and A_TW again.

Pardosa agricola (Thorell 1856)

Material: SWEDEN: *Dalarna*, Ore, lake Skattungen, sandy shore with reed debris, 1974–1979, $n = 5$, T. Kronestedt (NHRS).

Literature: Kronestedt (1979a).

Description of the display: The male starts waving the palps (P_W) in a down-flexed position, performing A_TW and L_O at the same time (Fig. 36). Forelegs are raised so that femora are directed upward and patellae-tarsi are directed forward, exposing the blackened tarsi. When approaching the female, the male moves his palps, tips directed towards the substrate.

Pardosa blanda (C.L. Koch 1833)

Material: ITALY, *Piedmont*, province of Cuneo, Acceglio, Sorgenti del Maira, alpine meadow (1566m a.s.l.), 2 June 2009, $n = 7$, M. Isaia (RBINS); province of Cuneo, Melle, Colle della Ciabra, alpine meadow (1723m a.s.l.), 24 May 2009, $n = 8$, M. Isaia (RBINS).

Literature: none.

Description of the display: The male follows the female, performing P_Q and A_Q (duration: 0.52 ± 0.21 sec, 34 observations, $n = 5$ individuals). When the female stops, the male performs about six hops (B_H, number of hops: 6 ± 4 times, 48 observations, $n = 5$ individuals) toward the female (duration of a single hop: 0.67 ± 0.23 sec, 76 observations, $n = 5$ individuals). B_H starts with a step forward toward the female with L_WH. Immediately after, the male performs about two (2 ± 1 times, 72 observations, $n = 5$ individuals) small steps with P_UDJ, L_T and A_TA. If the female runs away, the male lifts the prosoma, performing A_TW and P_W once.

Pardosa mixta (Kulczyński 1887)

Material: ITALY: *Piedmont*, province of Cuneo, Vernante, Palanfrè, alpine meadow (1340m a.s.l.), 30 June 2010, $n = 9$, A. Chiarle (RBINS).

Literature: none.

Description of the display: When the female is not moving, the male starts performing cautious B_SW movements toward her (duration of each wave: 1.53 ± 0.75 sec, 73 observations, $n = 5$ individuals). Getting closer to the female, the male performs L_R (duration: 0.20 ± 0.10 sec, 43 observations, $n = 5$ individuals). If the female remains on the spot, the male performs P_ARJ and after that suddenly leaps toward her, performing L_WH against her body (duration: 0.25 ± 0.05 sec, 57 observations, $n = 5$ individuals). Simultaneously, the male starts P_Q and A_TA. The legs are kept high, stretched almost perpendicular to the substrate, exhibiting the hairiness on the tarsus, metatarsus, and tibia. While keeping this position, the male starts P_ARJ and A_TW. If the female remains motionless, the male hits the female with L_WH. If the female runs away, the male repeats P_ARJ and A_TA, alternating it with B_SW until he reaches the female again.

Pardosa monticola (Clerck 1757)

Material: SWEDEN: *Uppland,* Runmarö, Vitträsk, 11 May 1974, $n = 1$, T. Kronestedt (NHRS); Vallentuna, Örsta, rock with lichens, 6 June 1976, $n = 3$, T. Kronestedt (NHRS); Bohuslän, Hamburgön, rocky habitat at the sea, 26 May 1979, $n = 1$, T. Kronestedt (NHRS).

Literature: Kronestedt (1979a).

Description of the display: The male remains still on the spot, performing a single abdominal twitch (A_TW) now and then. During A_TW, the male quickly raises one of the palps upward and forward (Fig. 37) and lowers it slowly (P_RL). After a few more A_TW, the male raises the other palp and slowly lowers it (P_RL). As an example, the time interval between each P_RL was 12, 15, 15, 21, and 47 sec. Finally, the male rapidly approaches the female.

Pardosa palustris (Linnaeus 1758)

Material: SWEDEN: *Uppland,* Vallentuna and Täby, May–June 1974–1979, $n = 4$, T. Kronestedt (NHRS); *Dalarna*, Ore, Näset, 25 June 1978, $n = 1$, T. Kronestedt (NHRS).

Literature: Kronestedt (1979a).

Description of the display: The male moves slowly toward the female performing B_SW (Fig. 38). At the same time he performs P_W in a down-flexed position and A_TW, now and then.

Pardosa purbeckensis F.O.P.-Cambridge 1895 **stat. rev.**

Material: NETHERLANDS: *Friesland*, Lauwerszeepolder, 30 April 1974, $n = 6$, T. Kronestedt (NHRS).

Literature: Bristowe (1929), Kronestedt (1979a).

Description of the display: The male starts courting by raising the palps synchronously in one to three jerky movements (tips of palps upwards and sideways) (P_SWR). The male then raises the forelegs (L_R) and directs them forward and upward, obliquely (Figs. 30, 31). The palps are then lowered in synchrony and moved up and down (tips directed downwards) while the front legs, directed forward, oscillate rapidly (L_O) (Fig. 32). Abdominal twitching may occur (A_TW). The movements are performed on the spot, and sometimes the male leans his body forward toward the female. The entire sequence may be repeated several times.

Based on these observations and from arguments given above, we propose the re-establishment of *P. purbeckensis* as a distinct species.

Pardosa torrentum Simon 1876

Material: ITALY: *Piedmont*, province of Verbania Cusio Ossola, Fondo Toce – Riserva Naturale Speciale di Fondo

Toce, artificial lake shore (193m a.s.l.), 6 April 2009, $n = 10$, A. Chiarle & M. Paschetta (RBINS).

Literature: none.

Description of the display: When the male approaches the female, he starts A_TW, followed almost immediately by A_TA. This behavior turns into B_SW (number of consecutive oscillations: 21 ± 16 times; duration of a single oscillation: 0.40 ± 0.12 sec, 89 observations, $n = 5$ individuals) with L_O. The legs are raised obliquely and slowly and rhythmically oscillated. At the same time, the palps (down-flexed at the beginning) are moved up and down, keeping them in contact with the substrate (P_UDJ). While performing these movements, the male slowly approaches the female.

Pardosa nigra group

The *P. nigra* group has a Holarctic distribution and is morphologically distinct from other *Pardosa* species in having the male palp with tegulum strongly protruding ventral and a terminal apophysis characteristically connected to the palea. (Tongiorgi 1966a; Lowrie & Dondale 1981; Kronestedt 2004). Five species occur in Europe: *P. eiseni* (Thorell 1875), *P. giebeli* (Pavesi 1873), *P. lasciva* L. Koch 1879, *P. nigra* (C.L. Koch 1834) and *P. trailli* (F.O.P.-Cambridge 1873). This is the first account of courtship behavior in a species of this group.

Pardosa nigra (C.L. Koch 1834)

Material: ITALY: *Piedmont*, province of Torino, Mompantero, Rocciamelone, Alpine scree (3538m a.s.l.), 30 June 2007, $n = 5$, M. Isaia (MRSN).

Literature: none.

Description of the display: The male bends his body at the pedicel and performs P_C (duration: 0.38 ± 0.24 sec, 75 observations, $n = 5$ individuals) continuously, tips directed downward. At the same time, he performs L_R. These movements occur on the spot. After about three P_C (3 ± 2 times, 60 observations, $n = 5$ individuals), the male performs B_H and L_WH toward the female (duration of a single hop: 0.40 ± 0.10 sec, 100 observations, $n = 5$ individuals). At the end of each B_H, the male again starts P_C on the spot.

Pardosa nigriceps group

The *P. nigriceps* group encompasses two species (Hippa & Mannila 1982), both occurring in Europe: *Pardosa maisa* Hippa & Mannila 1982 and *P. nigriceps* (Thorell 1856). The courtship behavior of *P. nigriceps* has already been described in the literature, but no description is available for *Pardosa maisa*.

Pardosa nigriceps (Thorell 1856)

Material: BELGIUM: *Namur*, Viroinval, Nismes, meadow, 7 June 2009, $n = 10$, F. Hendrickx (RBINS). SWEDEN: *Uppland*, Täby, 10 May 1983, $n = 2$, T. Kronestedt (NHRS).

Literature: Locket (1923), Bristowe & Locket (1926), Bristowe (1958), Vlijm & Dijkstra (1966).

Description of the display: The male starts with slow movements toward the female with cautious steps and performing continuous palpal cycling (P_CC) (10 ± 8 times in one courtship session; one P_CC duration: 2.80 ± 1.05 sec, 73 observations, $n = 5$ individuals) and abdominal twitching (A_TW). When he gets closer to the female, he performs L_Q.

Pardosa proxima group

According to Zyuzin (1979), the *P. proxima* group comprises eight species, all confined to the Palearctic region. *Pardosa proxima* was originally described from Greece (Koch 1847). This species is widespread in open habitats in the Mediterranean basin (Den Hollander & Dijkstra 1974). The individual variability of *P. proxima* is very high, even within the same population, both in genital structures and general morphology (Tongiorgi 1966a). One subspecies, *P. p. poetica* Simon 1876, is still considered valid though, according to Tongiorgi (1966a); the validity of subspecies of *P. proxima* needs reconsideration. The first observations of the courtship behavior of *P. proxima* were given in Den Hollander et al. (1972). In the same work *P. vlijmi* was described as a new "ethospecies" from France. According to Den Hollander & Dijkstra (1974) and confirmed by our findings; *P. proxima*, *P. vlijmi*, and *P. hortensis* may occur in syntopy.

Pardosa hortensis (Thorell 1872)

Material: ITALY: *Piedmont*, province of Cuneo, Guarene, Sotteri, meadow (155m a.s.l.), 7 March 2009, $n = 8$, A. Chiarle (RBINS); province of Cuneo, Diano d'Alba, Gaiole Rinaldi, vineyard (275m a.s.l.), 9 July 2007, $n = 5$, A. Chiarle (MRSN).

Literature: Vlijm & Dijkstra (1966).

Description of the display: The male starts P_SC (number of steps: 4 ± 1 times each semicircular movement; one step duration: 0.78 ± 0.61 sec, 70 observations, $n = 5$ individuals; one P_SC duration: 4.08 ± 1.61 sec, 35 observations, $n = 5$ individuals). During P_SC, the male also performs L_Q, while stepping cautiously toward the females. Sporadic A_TW may occur.

Pardosa proxima (C.L. Koch 1847)

Material: ITALY: *Piedmont*, province of Cuneo, Guarene, Sotteri, meadow (155m a.s.l.), 22 March 2009, $n = 11$, A. Chiarle (RBINS).

Literature: Den Hollander & Dijkstra (1974), Chiarle & Isaia (2013).

Description of the display: The male moves a step forward (step duration: 0.39 ± 0.12 sec, 40 observations, $n = 6$ individuals) while performing L_R, P_W and A_TW. The male then moves toward the female with hops (B_H, mean number of hops = 5 ± 3 times; B_H, duration of single hop: 1.44 ± 0.35 sec, 215 observations, $n = 6$ individuals), while performing L_T, P_CR and A_TW. If the female runs away, the male raises its body and performs P_CR.

Pardosa vlijmi Den Hollander & Dijkstra 1974

Material: ITALY, *Piedmont*, province of Cuneo, Vicoforte Mondovì, Ermetta, meadow (534m a.s.l.), 07 March 2009, $n = 2$, A. Chiarle (RBINS); Guarene, Sotteri, meadow (155m a.s.l.), 22 March 2009, $n = 5$, A. Chiarle (RBINS).

Literature: Den Hollander & Dijkstra (1974), Chiarle & Isaia (2013).

Description of the display: The male starts B_B. The vibration turns into a conspicuous B_H, characterized by up and down movements of the whole body toward the female (mean number of hops: 9 ± 3 times; single hop duration: 0.60 ± 0.18 sec, 202 observations, $n = 6$ individuals). More specifically, the cephalothorax is raised due to the movements

of the posterior legs (second, third and fourth pairs), the first pair held near the body, parallel to the palps. At the same time, the male performs A_TW. In between B_H, palps and abdomen touch the substrate (P_CR and A_TA). If the female runs away, the male raises its body and performs P_CR.

Pardosa pullata group

The *P. pullata* group includes eight species and one subspecies, all found in the Palearctic region (Kronestedt 2007). The monophyly of this group is supported by morphology (division of the tegular apophysis into two membranously connected sclerites), as well as by molecular data (Zehethofer & Sturmbauer 1998; Goodacre & Kronestedt unpublished data). Some species have at times been treated as subspecies. Following Platnick (2012), only *P. prativaga scoparia* Simon 1937, reported from France, is still eonsidered valid.

Regarding courtship behavior, the *P. pullata* group is one of the most studied. Precopulatory behavior in *P. pullata* has been the subject for several studies (Bristowe & Locket 1926; Vlijm & Borsje 1969; Hallander 1967; Den Hollander 1971; Den Hollander al. 1973; Kronestedt 1979a, 2007). The different descriptions are congruent with each other, illustrating an inconspicuous visual display: the male rapidly jumps onto the female (B_J) and clasps her with its legs.

The behavior of *P. pyrenaica*, observed by Kronestedt (2007), is very similar to that of *P. pullata*, but, in addition, after the contact with the female, the male performs some alternating P_W. *Pardosa prativaga* and *P. sphagnicola* have been studied extensively by Den Hollander (1971: *P. prativaga fulvipes* = *P. sphagnicola*), Den Hollander et al. (1973) and Kronestedt (1979a).

Pardosa fulvipes (Collett 1876)

Material: SWEDEN: *Dalarna*, Ore, grassland, 3 June 1973, $n = 1$, T. Kronestedt (NHRS); *Uppland*, various localities in the vicinity of Stockholm, grasslands with thick cover of last year's grass debris, some adjacent to lakes, 1972–1987, $n = >20$, T. Kronestedt (NHRS).

Literature: Kronestedt (1979a).

Description of the display: The male initially takes up a posture with the first legs lifted up and hanging in front (Fig. 39). The two palps are then raised and lowered in alternation a few times (P_RL, duration of one palpal raising-lowering: 1.20 ± 0.10 sec, 10 observations, $n = 3$ individuals) (Fig. 40). This is followed by the raising and slowly lowering of the foreleg on the same side as the last lowered palp (L_WA, duration of one leg waving: 9.20 ± 1.20 sec, 12 observations, $n = 3$ individuals) (Fig. 41). During lowering, the leg is slowly waved with very slight bendings at the tibio-metatarsal joint. The thin hairs on the metatarsus are exposed during L_WA. When the foreleg touches the substrate, the body may jerk slightly and the palp on the opposite side is raised and lowered, followed by L_WA on the same side. During the display, the male performs small vertical twitches of the abdomen (A_TW), generating vibratory sensations from a paired stridulatory apparatus, barely heard by human ear when performed on an artificial substrate like cardboard (Kronestedt 1973).

Pardosa prativaga (L. Koch 1870)

Material: ITALY: *Piedmont*, province of Cuneo, Vicoforte Mondovì, Ermetta, meadow (534m a.s.l.), 21 March 2007, $n = 7$, A. Chiarle (MRSN). SWEDEN: *Uppland*, various localities in the vicinity of Stockholm, lake shores and adjacent grasslands, 1972–1983, $n = >25$, T. Kronestedt (NHRS).

Literature: Den Hollander et al. (1973), Kronestedt (1979a).

Description of the display: The male lowers its body, almost touching the substrate, then starts to vibrate the abdomen up and down (A_TA). This is very shortly followed by a few synchronous movements of the palps accompany the vibration. During this movement, the palps are held in a low position with their tips directed backward under the body (Fig. 42). After the very first vibrations, the abdomen taps on the substrate about 10 times (number of taps in one sequence: 10 ± 1 times, 20 observations; duration of one sequence with 10 taps: 1.76 ± 0.09 sec, 12 observations; interval between taps: 0.22 ± 0.01 sec, 96 observations, first shorter and weaker taps in a sequence not included; $n = 5$ individuals). This is followed by rapid synchronous up and down movements of the palps, cymbia now being directed downwards (P_Q) (duration of P_Q: 2.82 ± 0.48 sec, 87 observations, $n = 5$ individuals, Fig. 43). During the latter movements, the eymbia barely reach the substrate. This is usually followed by repeating A_TA with P_CR and P_Q a number of times until the male approaches the female in a series of B_H. During such hops, the male may perform A_TA and P_CR (number of hops varies according to the receptivity of the female, up to about 40 taps/hops have been recorded; interval between taps: 0.19 ± 0.02 sec, 130 observations, $n = 5$ individuals). Hops cease when the male is close to the female. Then the male approaches the female with raised first legs, tibiae parallel to the substrate and metatarsi perpendieular to the tibiae (Fig. 44). If the female moves away, the male quickly moves backward (B_R) (duration: 0.94 ± 0.27 sec, 32 observations, $n = 5$ individuals), performing a few abdominal taps. The whole behavior is repeated several times until copulation. Before starting a sequence, the male may approach the female and quickly touch her, then move baekwards (B_R) and perform encircling (B_E) (duration = 3.35 ± 1.39 see, 23 observations, $n = 5$ individuals). Sounds from A_TA produced on cardboard as substrate are illustrated in Fig. 48.

Pardosa riparia (C.L. Koch 1833)

Material: SWEDEN: *Dalarna*, Ore, various grasslands, 1977–1987, $n = 10$, T. Kronestedt (NHRS).

Literature: Kronestedt (1979a).

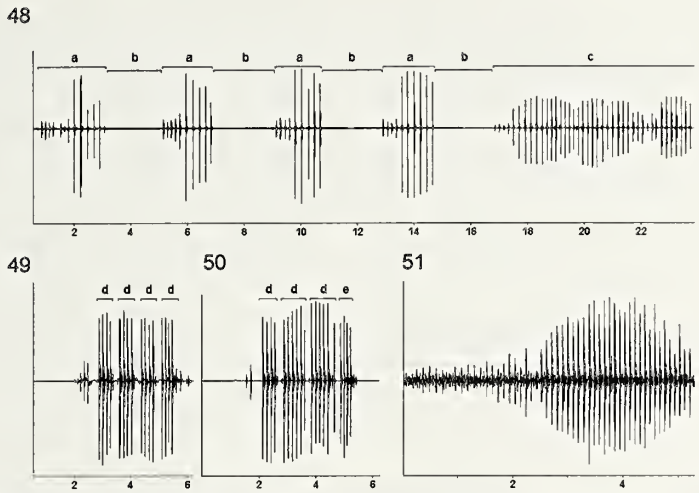
Description of display: There is no stereotypic visual component in the courtship. The male performs B_P intensely and tries to mount the female after a more or less intense struggle. When losing contact with the female, the male performs P_W and A_TW.

Pardosa sphagnicola (Dahl 1908)

Material: SWEDEN: *Dalarna*, Ore, various places, mires with *Sphagnum*, 1973–1981, $n = 5$, T. Kronestedt (NHRS); *Uppland*, various places in the vicinity of Stockholm, mires with *Sphagnum*, 1972–1983, $n = 13$, T. Kronestedt (NHRS).

Literature: Den Hollander et al. (1971: sub *P. prativaga fulvipes*, 1973), Kronestedt (1979a).

Description of the display: The male starts courting by performing vertical movements of the abdomen (A_TW) and synchronous movements of the palps, tips bent backward close



Figures 48–51.—Oscillograms illustrating the sound produced by abdominal tapping on cardboard during courtship in *Pardosa* species (time scale in seconds). 48. *P. prativaga*: sequence of four tapping groups (a) followed by tapping during hops towards female (c). Rapid up and down jerks of the palps (with no recorded acoustic effect: (b) are performed between each group of abdominal tapping. (a) and (b) are performed on the same spot; 49–50. *P. sphagnicola*: 49. Hopping sequence of four abdominal tapping groups (d) performed during approach towards female; 50. Hopping sequence of three abdominal tapping groups (d) performed during approach towards female and one group during retreat (e); 51. *P. schenkeli*: single abdominal drumroll (out of several) (51. from Kronestedt 2005).

to the body (P_Q). These movements increase in intensity, leading to a series of rapid hops (B_H, Figs. 45, 46), during which abdomen and forelegs tap the substrate (A_TA and L_T), and palps are held low with cymbia reaching the substrate (P_CR). B_H are fast and often grouped in about two to four (number of taps/hops in a sequence: 3 ± 1 times, 40 observations; time interval between taps/hops: 0.15 ± 0.02 see, 92 observations, $n = 6$ individuals), separated by a short break (time interval between tapping sequences: 0.32 ± 0.06 sec, 30 observations, $n = 6$ individuals). Hops cease when the male is close to the female and approaches her with first legs raised, tibiae being held parallel to the substrate and metatarsi perpendicular to the tibiae (Fig. 47). If the female runs away, the male quickly moves backwards (B_R) with A_TA and repeats the entire sequenee. Sounds from A_TA produced on cardboard are illustrated in Figs. 49, 50.

Pardosa wagleri group

Four species belonging to this group (Zyuzin 1979) occur in Europe, but only the courtship behavior of *P. wagleri* and *P. saturatior*, both occurring in riverine habitats, has been studied. *Pardosa wagleri* is widely distributed in low altitude riverbanks, while *P. saturatior* is restricted to higher altitudes (Tongiorgi 1966a). Despite the great similarity of the genitalia, Tongiorgi (1966a) separated these two species on ecological, phenological, and morphological basis. Barthel & von Helversen (1990) confirmed Tongiorgi's (1966a) observations, adding preliminary information on differences in courtship behavior.

Chiarle et al. (2010) described and compared the courtship behavior of *P. wagleri* and *P. saturatior*. In both species, jerky movements of the palps (P_LJ for *P. wagleri* and P_Q for *P. saturatior*) characterize the first part of the behavior, while performing L_WH, A_Q and A_TA. The male then starts B_P while performing all previous behaviors. *Pardosa saturatior* exhibits faster and more intense movements in its behavior than *P. wagleri*. The last part of the behavior, however, is identical, characterized by P_RL.

GENERAL CONSIDERATIONS

Movements mainly involving three body parts eharacterize courtship display in the genus *Pardosa*: the palps, the first pair of legs, and the abdomen. The most complex movements are performed by the palps, involving all joints in different movements. Legs are mainly oscillated in the air or vibrated on the substrate, while the abdomen is generally moved up and down with different speeds and amplitudes.

A considerable diversity in visual signaling behavior is shown both within and among the so-called "phenetic groups." Even if they do not refleet real phylogenetic groups, species-groups are still widely used for practical purposes in arachnological literature (Zyuzin 1979; Almquist 2005).

Despite strong morphological similarities in genitalia and habitus, species belonging to the *P. monticola* group show clear qualitative differences in courtship behavior. The general body movement of *P. blanda* is quite different from all the other species in the group, characterized by the male's hop-like movements towards the female. In contrast, *P. torrentum*, *P. mixta,* and *P. palustris* perform B_SW, while the other species display on-the-spot movements or move slowly toward the female. The movements of the palps are complex in all the species that we analyzed, especially in *P. agrestis*, *P. purbeckensis*, *P. mixta,* and *P. agricola*. The position of the front legs during the display is similar in *P. agrestis*, *P. torrentum* and *P. agricola*. It is worth noticing that the coloration of the tarsal forelegs may be related to such behavior, playing a role in the visual signal associated with the movements. Similarly, some other epigamic or amplifying traits in this group are found in the divergent hairiness on foreleg tarsi and metatarsi of *P. purbeckensis* and *P. mixta*, the latter lifting the forelegs high, exhibiting the hairiness on its metatarsi and tarsi to the female. As observed in the genus

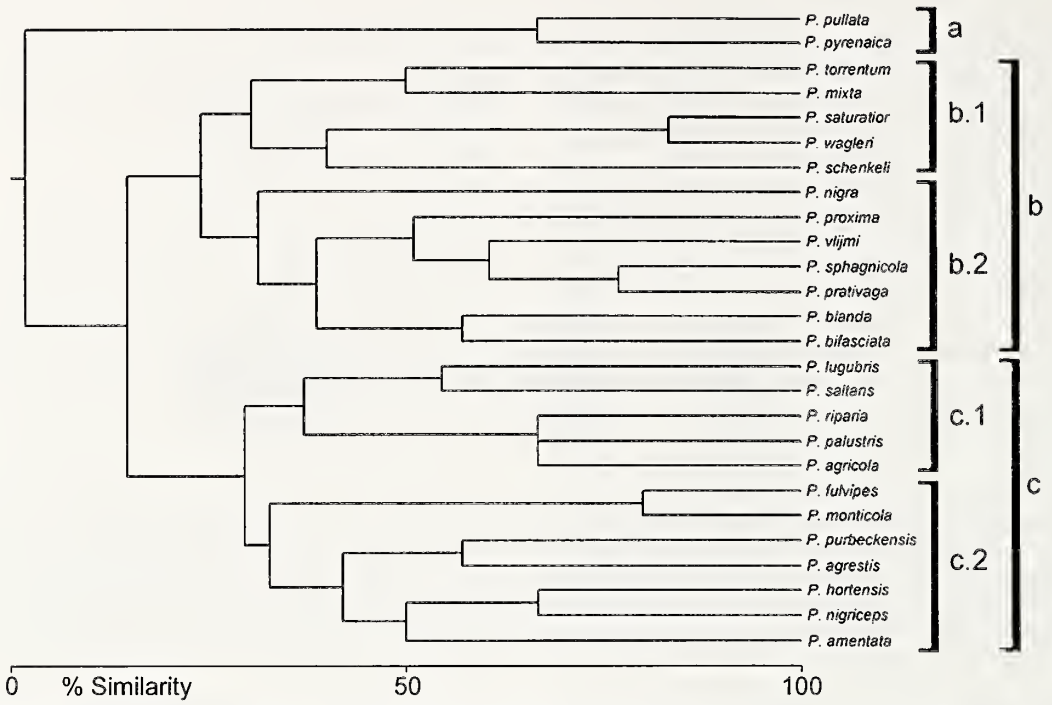


Figure 52.—Cluster analysis showing the degree of association within the species of *Pardosa* here considered (presence/absence data, group average link rule, Bray Curtis distance). Letters indicate groups discussed in the text.

Schizocosa, dark forelegs and tufts of hairs on the legs generally represent amplifying traits related to male qualities (McClintock & Uetz 1996; Scheffer et al. 1996; Hebets & Uetz 1999, 2000).

Among the species of the *P. pullata* group, the display of *P. fulvipes* (P_RL, L_WA and A_TW) shows no similarities to any other species of the group. Moreover, *P. fulvipes* uses a specific and unique stridulatory organ on the abdomen: the hairless striated surface of the book-lung opercula serves as the file, while the stout denticulated hairs on the fourth coxae form the scraper part (Kronestedt 1973). Despite the general complexity of the display observed in this group, three species show inconspicuous behaviors: *P. pullata*, *P. pyrenaica* and *P. riparia* simply jump on or chase the female (B_J and B_P). The elosely related *P. prativaga* and *P. sphagnicola* show comparable behaviors, with the presence of common traits: in both species the male approaches the female with a series of hop-like movements (B_H), characterized by tapping of the abdomen (A_TA) against the substrate, thus producing a percussive sound (Figs. 48–50).

Similar considerations can be drawn for the *P. proxima* group, in which the cryptic species *P. proxima* and *P. vlijmi* perform different behaviors. However, several hypothetical homologies, such as the general courtship patterns, B_H and P_CR are easily identifiable. *Pardosa hortensis*, belonging to the same group, performs P_SC, L_Q and A_TW, in contrast to the fast B_H in *P. proxima* and *P. vlijmi*.

Concerning the *P. wagleri* and *P. lugubris* groups, extensive and quantitative data are reported in Chiarle et al. (2010) and Töpfer-Hofmann et al. (2000) respectively. We refer to those works for the detailed description of the courtship displays. It is worth noticing that the presence of an identical ending behavior (P_RL) in *P. wagleri* and *P. saturatior* can be interpreted as a homology. Similarly, *P. lugubris* and *P. saltans*, though performing dissimilar behavior, share some identical CEs (L_LS and L_P).

COMPARATIVE ANALYSIS

Despite the fact that all categorizations are naturally subject to personal interpretation, we explored the degree of association between the European species of the genus *Pardosa* using a cluster analysis. The dataset was set up on the basis of the CEs previously described. We used the Bray-Curtis distance (group average link rule) to evaluate similarities among species and clustered them according to presence/absence of CEs within the display. The final matrix was thus composed of 35 lines (CEs) and 26 columns (species).

Pardosa prativaga, *P. sphagnicola*, and *P. blanda* show the highest number of CEs within the display ($n = 9$) followed by *P. mixta*, *P. sphagnicola*, and *P. saltans* ($n = 7$). *Pardosa pullata* ($n = 1$), *P. pyrenaica*, and *P. monticola* ($n = 2$) show the poorest displays in terms of the number of behavioral elements. A_TW ($n = 18$), A_TA ($n = 9$), and B_H ($n = 7$) and P_W ($n = 7$) had the most frequent CEs among the species. In general, palpal movements resulted in highly species-specific behaviors (P_SE, P_C, P_LWJ, P_P, P_ARJ, P_SWR, P_LJ, P_SRL, P_TRL, P_SC, P_CC). A similar trend occurred for leg movements such as L_P and L_LS (characteristic only of the *P. lugubris* group) and peculiar body movements such as B_R (shared by the two closely related species *P. sphagnicola* and *P. prativaga*) and B_J (shared by the two closely related species *P. pullata* and *P. pyrenaica*).

The cluster analysis (Fig. 52) highlights the partitioning of the species in three main clusters, based on the presence of different CEs within the displays. Group "a" is composed of two species (*P. pullata* and *P. pyrenaica*) performing simple visual displays characterized by one or two CEs (P_W and

B_J). Group "b" is characterized by species performing rapid, directional movements toward the female (B_H, B_B, B_P, B_R, B_E). This cluster could be further subdivided into two subgroups. The first subgroup (b.1) is composed of three species (*P. wagleri*, *P. saturatior*, and *P. schenkeli*) that perform relatively fast movements of the palps (P_Q, P_P, P_RL, P_LJ), while the legs are whipped (L_WH) and the abdomen is quivered (A_Q) or tapped (A_TA) against the substrate. The second sub-cluster (b.2) includes seven species (*P. nigra*, *P. proxima*, *P. vlijmi*, *P. sphagnicola*, *P. prativaga*, *P. blanda*, and *P. bifasciata*), which perform hopping behavior (B_H). Palpal movements within this subgroup are mainly characterized by quivering (P_Q) or waving (P_W). In addition, cymbium rubbing (P_CR) may occur. Group "c" encompasses all the remaining speeies, and in this case two subgroups can be highlighted. The first subgroup (c.1) comprises the two closely related species *P. lugubris* and *P. saltans*, three species of the *P. monticola* group (*P. agricola*, *P. monticola*, and *P. palustris*) and two species of the *P. pullata* group (*P. fulvipes* and *P. riparia*). This cluster is rather heterogeneous, and thus no clear characteristics can be highlighted. In general, the displays are predominantly slow and cautious, with faster final stages. The last subgroup (c.2) encompasses seven species (*P. agrestis*, *P. amentata*, *P. hortensis*, *P. mixta*, *P. nigriceps*, *P. purbeckensis* and *P. torrentum*), whose males generally court on the spot or approach the female with small steps. The movements of the palps are, however, highly species-specific, characterized by up and down movements (P_UDJ, P_SWR, P_Q) and semi-circular movements (P_ARJ, P_LWJ, P_SE, P_SC, P_CC). Leg quivering (L_Q) and leg oscillation (L_O) is generally associated with the movement of palps while the abdomen is twitched (A_TW), occasionally during the whole courtship. Only *P. mixta* and *P. torrentum* perform abdomen tapping (A_TA).

Aecording to our analysis, the species do not show high levels of similarity due to the species-specificity of each courtship display. However, it is possible to highlight some hypothetical homologies in certain closely related species. Some examples are provided by *P. torrentum*, *P. palustris*, and *P. mixta* (B_SW), *P. sphagnicola* and *P. prativaga* (shared CEs: P_Q, P_CR, A_TA, B_H), *P. saturatior* and *P. wagleri* (shared CEs: P_RL, L_WH, A_TA, A_Q, B_P), *P. pullata* and *P. pyrenaica* (shared CEs: B_J, unique for these two species) and *P. lugubris* and *P. saltans* (shared CEs: P_TC, A_TW, L_LS). As reported by Vlijm & Dijkstra (1966), it is interesting to note that *Pardosa hortensis*, *P. nigriceps* and *P. amentata* all belong to different morphological species groups, but show very similar courtship outlines.

CONCLUSIONS

Almost all the species that we observed showed a high level of complexity, both in terms of sensory modes involved and number of CEs comprising the display. A similar level of complexity was observed in some other lycosids (Stratton & Uetz 1983; Scheffer et al. 1996). As stated by Bull (1979) regarding the complex courtship of the grasshopper *Myrmeleotettix maculatus*, complexity in visual display might have arisen due to different mechanisms sueh as 1) an increase in male attractiveness in a heterogeneous environment; 2) reproductive isolation between closely related species upon seeondary contact; 3) sexual selection if males with higher display complexity have a greater fitness; and 4) sensory exploitation and female preference for complexity. Moreover, the organization of courtship is hierarchical, and it is possible to split the behavior into different semi-independent parts regarding their regulation, function and evolution. All of these features facilitate independent variation of different CEs, increasing diversification and complexity. Despite the observed general complexity, in five out of the nine groups studied in this work, we found species with simple courtship behavior: *P. pullata*, *P. pyrenaica*, *P. riparia*, *P. lugubris*, *P. bifasciata* and *P. nigra*. If conspicuous traits lose their previous importance, selection should act by reducing costly courtship display regarding both energy expenses and conspicuousness to rival males or other predators (Bull 1979).

In conclusion, courtship behavior in genus *Pardosa* is very diversified, and in several cases it is hard to reconstruct the path that leads to a particular courtship within the same morphology-based species group. Furthermore, it is also possible to find similar or identical behavior in species that are not closely related, such as the hopping behavior in both *P. proxima* (*P. proxima* group) and *P. blanda* (*P. monticola* group) or the elliptical movements of the palps in *P. hortensis* (*P. proxima* group) and *P. nigriceps* (*P. nigriceps* group).

For these reasons, the study of eourtship behavior represents a useful tool for identifying cryptic species due to the qualitative differences that can be observed in closely related species (see Den Hollander & Dijkstra 1974 and Töpfer-Hofmann et al. 2000). However, courtship behavior should not be used solely to infer phylogenetie relationships among species as highlighted by the cluster analysis.

Further studies on this topic are thus needed to increase the knowledge of both the biology of *Pardosa* species and the evolution of such complex behaviors. In parallel with the genus *Schizocosa* (see McClintock & Uetz 1996; Scheffer et al. 1996; Hebets & Uetz 1999, 2000; Uetz 2000; Taylor et al. 2005; Delaney et al. 2007; Gibson & Uetz 2008; Vaccaro et al. 2010), *Pardosa* models may provide useful tools to study the evolution of communication and behavioral complexity.

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Are phenological patterns of ballooning spiders linked to habitat characteristics?

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Abstract. We describe here the phenological patterns of the 25 most common ballooning speeies of spiders caught by a 12.2 m suction trap during an eleven year survey in Switzerland. We aimed at identifying and quantifying the number, position, spread, and relative weight of activity periods for the whole community. Further, we explored the possible link between phenological patterns and habitat use. For this purpose, we used bump-hunting approaches and fitted mixtures of normal distributions to the abundance data. The phenologies can be grouped in four categories, from uni- to quadrimodal. The specific peaks in the timing of ballooning were found between February and November, with most ballooning activity occurring in summer and autumn. For some taxa, it was possible to analyze the data for young instars and adults. For the majority of taxa, the adults' peak appeared between the early and late peaks of immature individuals. Species inhabiting the ground level of open areas, often disturbed by agricultural practices, were clearly dominant in the multimodal categories; spiders living in more closed and stable habitats, such as tree-shrub and herb layers, typically had a single peak of adult dispersal. This discrepancy in phenology may simply reflect different numbers of generations, but may also result from an adaptation to maximize the persistence of populations in unstable habitats.

Keywords: Araneae, dispersal, habitat use, suction trap, Switzerland

Spiders have the capacity to travel by air, suspended by a silk thread that is used as a sail. This mode of dispersal, called ballooning, allows these organisms far-reaching colonization abilities (Bell et al. 2005). According to Marc et al. (1999), dispersal of spiders occurs 1) when the structure or microclimatic conditions of habitats change, 2) when competition is too high and 3) at particular periods in the life cycle: juveniles in transition from gregarious to solitary phase and adults during reproductive periods. Dispersal abilities of the different species are linked to habitat preferences and landscape configuration (Bonte et al. 2003b, 2006, 2010). These abilities can also vary among individuals of the same species according to their genetic background (Bonte et al. 2003a), thermal conditions during juvenile development (Bonte et al. 2008), perturbation of habitat (Entling et al. 2011), presence of microbial endosymbionts (Goodacre et al. 2009), inbreeding (Bonte 2009), food stress (Mestre & Bonte 2012) and information from other congeners (De Meester & Bonte 2010). In all cases, ballooning can be effective only if meteorological conditions are suitable for take-off (Reynolds et al. 2007).

According to Plagens (1986), the aerial dispersal phenology of spiders is linked to a change in the population density and to modifications of the carrying capacity of the species in the environment. Dispersal strategies are strongly linked with the biology of the species. The life cycle of almost all European Araneomorphae spiders lasts for one or two years, with a maximum of three years. Some Linyphiidae can have two to three generations of adults per year (De Keer & Maelfait 1987, 1988; Thorbek et al. 2003; Topping & Sunderland 1998). According to Marc et al. (1999), two main categories of life cycles are generally recognized: 1) spiders of the eurychronous type reproduce and disperse from spring to autumn and overwinter in different stages; 2) the stenochronous ones show precise reproductive and dispersal periods, with temperature and photoperiod regulating their cycles. Additionally, stenochronous spiders can be grouped into three types. First, the stenochrones of spring spend winter as immature instars, become adults in spring and summer and disperse in summer. They can also have two mating periods in spring and autumn (formerly called diplochronous). Second, the stenochrones of autumn lay their eggs during the autumn and have an obligatory diapause in the hibernation stage. And third, the stenochrones of winter reproduce in winter. In agroecosystems, Samu & Szinetár (2002) showed that agrobiont spiders have a life cycle synchronized with the arable-crop season.

In the study area, earlier results based on all taxa pooled showed that aerial dispersal occurs almost year-round and that ballooning activity has two main periods, in summer and autumn (Blandenier & Fürst 1998; Blandenier 2009). The aim of the present study was to investigate the phenology of ballooning at the species level and to understand its relationship with the ecology and habitat characteristics of the spiders. We adopted a community-level analysis using the 25 most abundant species. With 11 years of weekly samples, our data set belongs with the few other multiannual studies dealing with whole spider communities; moreover, the sampling size is large enough to allow an investigation of the adult stage. Note that the shift of phenologies over the study period will be the subject of another contribution.

METHODS

Ballooning spiders were collected at a height of 12.2 m by a Rothamsted Insect Survey suction trap (Taylor & Palmer 1972; Derron & Goy 1987). The trap was located in a fragmented agricultural landscape located in the western region of the Swiss Plateau (in Changins, Canton of Vaud, 46°24′8″N, 6°14′0″E, 440 m a.s.l., mean annual temperature: 10.8 °C, mean total amount of precipitation: 1091 mm per year during the study), at the research station Agroscope ACW Changins-Wädenswil. A short description of habitats in the area surrounding the trap can be found in Blandenier (2009).

Data were collected weekly for 11 years from 16 April 1994 until 31 December 2004. We stopped the operation of the trap

in winter at the beginning of the survey between 17 December 1994 and 17 Mareh 1995, and between 3 December 1995 and 17 March 1996. For maintenance, it was stopped between 12 February 1998 and 21 April 1998. Outside these periods, the trap was working continuously, representing a total of 519 sampled weeks.

Adult spiders were determined to species, and immatures to species, genus or family level. We identified some juveniles and penultimate adults to species if unequivocal (five Araneidae, two Thomisidae, one Lycosidae, one Anyphaenidae and one Theridiidae). A total of 15,398 spiders were trapped, belonging to 16 families and 103 species. The list of taxa and the ecologieal classification of the species have been published in Blandenier 2009. Phenological types (Table 1) follow Nentwig et al. (2010), Schaefer (1976) and Ysnel & Canard (1986).

For our analysis, we retained 25 species (21 adults and 4 immatures) with a total number of 20 or more individuals captured. This number corresponds to the minimum limit with which individual peaks could be confidently detected with visual inspection. For each taxon and week (w), we computed the sum of the abundances over the 11 years of the study (y_w). We handled missing data (during trap maintenance periods, see above) as follows: for each taxon, we replaced the missing data with the mean abundance for that week during the sampled years. Because absence of trapping occurred mostly during low activity periods of spiders, this correction had negligible impact on the results.

We used a "bump hunting" approach (Good & Gaskins 1980) to define the number of activity periods in the yearly phenology. We applied the method developed by Silverman (1981) to find the number of significant modes (or bumps) in a distribution. This method relies on kernel density estimation, which approximates, or smooths, an observed distribution by summing Gaussian curves with the same standard deviation (the bandwidth) placed at each observation. The idea of the approach is first to find eritical bandwidths, and secondly to estimate their significance. For a given number of peaks k, the critical bandwidth c_k is the minimum standard deviation that produces a kernel density estimate with k peaks. In our case, we considered one to five peaks. The significance of c_k is then tested with a parametric bootstrap: a bootstrapped distribution is constructed by drawing with replacement n random numbers from the corresponding kernel density estimate, with n being the number of observations. This bootstrapped distribution is then used to estimate again a bootstrapped critical bandwidth c^*_k for k peaks. We repeated this resampling 1000 times for each peak. The significanee of c_k is given by the proportion of c^*_k larger or equal to c_k for the observed distribution. A significant value (we ehose a significance threshold of 0.1) for k peaks indicates that the c_k for our observations is excessively large, in other words that our observed distribution has more peaks. The estimated number of peaks is given by the first non-significant value in the series of critical bandwidths for one, two, and up to five peaks. We wrote a script in R (R Development Core Team 2012) for this purpose (the code is available upon request to the corresponding author).

From the bump hunting results (Table S1 for all results, online at http://www.bioone.org/doi/suppl/10.1636/P12-48) we found that the annual abundance patterns of the 25 species can include one to four activity peaks (Fig. 1). For each peak, we described an activity period (i) by the position and spread of its "bump." Note that the bump-hunting approach does not provide an estimate of the spread and of the "importance" of a given peak, only of its position. For this reason, we chose to describe each activity period with a Gaussian eurve with mean m_i [days] (the position) and standard deviation s_i [days] (the spread). For this purpose, for each taxon we fitted a mixture of Gaussian curves to the weekly abundance data:

$$\hat{y}_w = \sum_{i=1}^{k} w_i \cdot N\left(m_i, s_i^2\right),$$

with k representing the number of peaks obtained by bump hunting (k = 1, 2, 3 or 4), and w_i representing the weight of each activity period ($\Sigma w_i = 1$). We used a maximum likelihood method for the estimation of parameters. Note that our phenological data are circular data in the strict sense, but they can be analyzed here as ordinary data because of the very low ballooning activity in December and January.

RESULTS

Peaks of aerial dispersal occurred between February and November, with most dispersal occurring in summer and autumn (Table 1). The mean spread of all activity periods of adults was 53 days, with autumn's peaks being the shortest with 38 days on average. The activity patterns of the 25 studied taxa can be grouped in four categories, from uni- to quadrimodal (Table 1). Typical examples are shown in Fig. 1. The raw data for all taxa with less than 20 captured individuals and the figures of activity patterns of the 25 studied taxa are provided as a table (Table S2, online at http://www.bioone.org/doi/suppl/10.1636/P12-48) and in figures (Figs. S1, S2 & S3, online at http://www.bioone.org/doi/suppl/10.1636/P12-48).

There was a strong link between the habitat of the spider and the number of peaks of dispersal activity. Species inhabiting open habitats at ground level showed more dispersal peaks (2, 3 or 4 during the year) than those inhabiting the upper strata of closed habitats eovered by trees and bushes (one peak). Comparing the frequencies of species with one versus species with more than one peak in open and closed habitats yielded a highly significant relationship (Fisher's exact test: $P < 0.001$).

Eight species (two Araneidae, two Philodromidae, two Linyphiidae, one Salticidae, and one Thomisidae) showed one main period of dispersal in the year (Fig. 1a and Table 1). The mean spread for these species was 42 days. Six species (75%) were found in the herb and/or tree layer and two lived at ground level. Seven were spring stenochrones, and one was an autumn stenochrone. For *Oedothorax fuscus* (Blackwall 1834), in spring nearly all ballooning spiders were females.

Four species (one Tetragnathidae, one Theridiidae and two Linyphiidae) showed a bimodal pattern (Fig. 1b and Table 1). The mean duration of peaks for these species was 60 days, with the second peak always being shorter. All species were from the ground level. They were all eurychrones. Activity periods had roughly equal weights for *Pachygnatha degeeri* Sundevall 1830, *Tenuiphantes tenuis* (Blackwall 1852) and *Robertus arundineti* (O.P.-Cambridge 1871). The autumn period was more important in *Mermessus trilobatus* (Emerton

Table 1.—Phenology of dispersal of 25 most abundant species. Legend: ad., imm., number of adults and immatures, respectively (numbers in italics are for adults that were not analyzed because of low abundances); st. stratum, *G* ground-living, *H* herb layer, *T* trees and bushes, ecol. Ecology, *M* meadows, *OA* open areas, *F* forests (> 30% canopy cover), *FI* fields, *DM* dry meadows, *BR* buildings (and rocks), *BU* bushes, *T* trees, phen. Phenology, *e* eurychrones, *ss* stenochrones of spring, *sa* stenochrones of autumn. For each activity period: *m* [day/month], *s* [d], *w* [%] are the mean, standard deviation and weight, respectively. Species: *Anyphaena accentuata* (Walckenaer 1802), *Aculepeira ceropegia* (Walckenaer 1802), *Araniella opisthographa* (Kulczynski 1905), *Mangora acalypha* (Walckenaer 1802), *Nuctenea umbratica* (Clerck 1757), *Zygiella x-notata* (Clerck 1757), *Araeoncus humilis* (Blackwall 1841), *Bathyphantes gracilis* (Blackwall 1841), *Mermessus trilobatus* (= *Eperigone trilobata*) (Emerton 1882), *Erigone atra* Blackwall 1833, *Erigone dentipalpis* (Wider 1834), *Meioneta mollis* (O.P.- Cambridge 1871), *Meioneta rurestris* (C.L. Koch 1836), *Meioneta simplicitarsis* (Simon 1884), *Oedothorax apicatus* (Blackwall 1850), *Oedothorax fuscus* (Blackwall 1834), *Porrhomma microphthalmum* (O.P.-Cambridge 1871), *Porrhomma oblitum* (O.P.-Cambridge 1871), *Tenuiphantes tenuis* (Blackwall 1852), *Philodromus aureolus* (Clerck 1757), *Philodromus rufus* Walckenaer 1826, *Salticus zebraneus* (C.L. Koch 1837), *Pachygnatha degeeri* Sundevall 1830, *Robertus arundineti* (O.P.-Cambridge 1871), *Diaea dorsata* (Fabricius 1777). Note that we provide the results for one and two activity periods for Mer tri (see Table S1 for explanation). Nomenclature follows Platnick (2013).

						Late Winter peak			Spring peak			Early Summer peak			Late Summer peak			Autumn Peak		
Family/Species	ad.	imm.	st.	ecol.	phen.	*m*	*s*	*w*	*m*	*s*	*w*	*m*	*s*	*w*	*m*	*s*	*w*	*m*	*s*	*w*
Anyphaenidae																				
Any acc	*11*	21	*T*	*T BU*	*ss*	.	.	.	.	.	.	26/7	83	100	.	.	.	.	.	.
Araneidae																				
Acu cer	*1*	42	*H*	*M*	*ss*	.	.	.	.	.	.	23/7	39	100	.	.	.	.	.	.
Ara opi	23		*HT*	*T BU*	*ss*	.	.	.	7/6	14	100	.	.	.	.	.	.	.	.	.
Man aca	*1*	125	*HT*	*M*	*ss*	.	.	.	24/4	19	10	.	.	.	.	.	.	27/9	35	90
Nuc umb	*5*	661	*T*	*T BU*	*e*	.	.	.	.	.	.	4/7	32	76	13/8	11	11	29/9	18	13
Zyg x-n	47		*T*	*BR T BU*	*sa*	.	.	.	.	.	.	.	.	.	11/9	26	100	.	.	.
Linyphiidae																				
Ara hum	706		*G*	*M FI*	*e*	18/3	30	11	.	.	.	29/6	18	34	.	.	.	23/10	21	55
Bat gra	159		*G*	*OA*	*e*	20/3	24	13	.	.	.	15/7	27	57	.	.	.	7/11	16	30
Mer tri	219		*G*	*M*	*e*	.	.	.	.	.	.	.	.	.	27/8	64	41	29/10	13	59
Mer tri	219		*G*	*M*	*e*	.	.	.	.	.	.	.	.	.	.	.	.	3/10	52	100
Eri atr	358		*G*	*OA FI*	*e*	26/2	30	16	.	.	.	13/7	32	61	.	.	.	25/10	25	23
Eri den	533		*G*	*OA FI*	*e*	.	.	.	24/3	34	6	18/6	15	25	9/8	15	33	27/10	18	36
Mei mol	28		*G*	*OA FI*	*e*	20/3	10	7	.	.	.	27/7	23	54	.	.	.	5/11	11	39
Mei rur	1228		*G*	*OA FI*	*e*	14/3	29	8	.	.	.	31/7	33	63	.	.	.	1/11	19	29
Mei sim	34		*G*	*DM*	*e*	18/3	30	11	.	.	.	29/6	18	34	.	.	.	23/10	21	55
Oed api	121		*G*	*OA FI*	*e*	18/3	14	18	.	.	.	24/6	9	51	2/9	55	30	.	.	.
Oed fus	21		*G*	*M FI*	*ss*	.	.	.	.	.	.	16/6	38	100	.	.	.	.	.	.
Por mic	541		*G*	*OA FI*	*e*	.	.	.	18/4	57	21	15/7	17	61	.	.	.	8/11	26	18
Por obl	33		*G*	*M*	*ss*	.	.	.	17/6	31	100	.	.	.	.	.	.	.	.	.
Ten ten	245		*G*	*OA*	*e*	.	.	.	.	.	.	28/6	38	48	.	.	.	10/10	29	52
Philodromidae																				
Phi aur	45		*HT*	*T BU*	*ss*	.	.	.	9/6	13	100	.	.	.	.	.	.	.	.	.
Phi ruf	67		*HT*	*T BU*	*ss*	.	.	.	26/5	14	100	.	.	.	.	.	.	.	.	.
Salticidae																				
Sal zeb	33		*T*	*T BU*	*ss*	.	.	.	30/5	18	100	.	.	.	.	.	.	.	.	.
Tetragnathidae																				
Pac deg	33		*G*	*M FI*	*e*	10/3	19	54	.	.	.	.	.	.	.	.	.	27/10	9	46
Theridiidae																				
Rob aru	48		*G*	*M*	*e*	.	.	.	31/5	51	56	.	.	.	.	.	.	1/11	19	44
Thomisidae																				
Dia dor	27		*HT*	*T BU*	*ss*	.	.	.	21/5	13	100	.	.	.	.	.	.	.	.	.

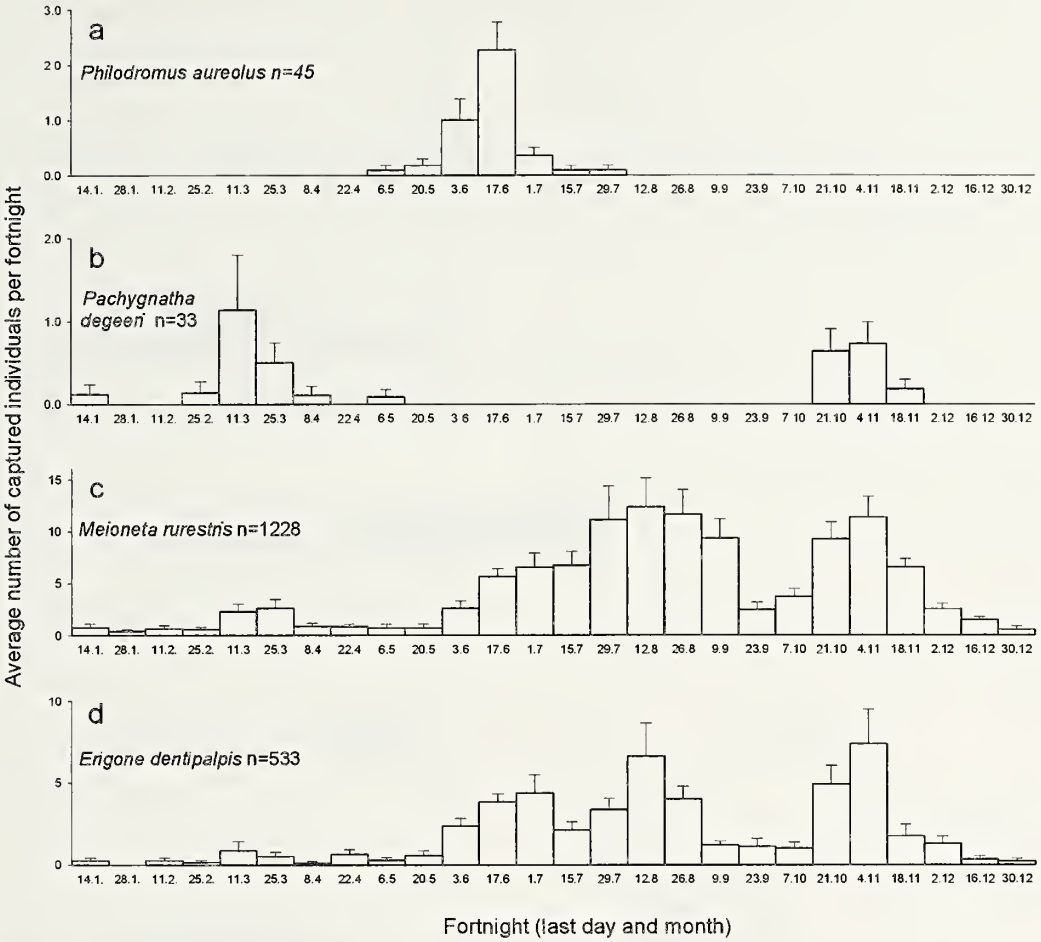

Figure 1.—Examples of types of ballooning phenology (mean and standard deviation of the number of captured individuals per fortnight between 1994 and 2004): unimodal species, *Philodromus aureolus*; bimodal species, *Pachygnatha degeeri*; trimodal species, *Meioneta rurestris*; quadrimodal species, *Erigone dentipalpis*.

1882). The interval between peaks was between 63 and 231 days.

Eight species (all Linyphiidae) showed three main periods of ballooning (Fig. 1c and Table 1). The mean duration of peaks for these species was 50 days, with the first one usually being the longest, as in the extreme case of *Porrhomma microphthalmum* (O.P.-Cambridge 1871). The pattern was unusual for *Oedothorax apicatus* (Blackwall 1850), for which the third period was the longest one. All these species lived at ground level in open habitats and were eurychrones. The summer and autumn periods had the largest weight for six and two species, respectively. In all cases, the first period in late winter consisted of only a few individuals. Sexual differences were apparent in *Araeoncus humilis* (Blackwall 1841), with the first period composed predominantly of females, and in *Oedothorax apicatus*, in which females dominated in first and third periods. The mean interval between the first and the second peaks was 114 days and the interval was 104 days between the second and the third peaks.

Erigone dentipalpis (Wider 1834) (Linyphiidae) was the only species with four identifiable periods of ballooning (Fig. 1d and Table 1). The mean duration of peaks for this species was 41 days, with the first peak being the longest, but the autumn period having the largest weight. This eurychrone species lives at ground level in open habitat. On average, the interval between the peaks was 72 days.

It was possible to reliably identify the adult and juvenile/immature stages of seven taxa. In general, more young were captured (Fig. 2 and Table 1). Although adults had only one peak, juveniles and immatures could have up to three. The peak of adults generally followed the juveniles' first peak and appeared before the juveniles' last peak. In the case of *Zygiella x-notata* (Clerck 1757), ballooning of adults occurred at the end of the season, after the ballooning of juveniles and immatures.

DISCUSSION

Our study of ballooning phenology reveals a strong link between the number of activity periods for adults' dispersal and habitat types. Species with multimodal distributions all inhabit the ground level of open habitats, and species with a unimodal pattern are mostly found in closed habitats. We were also able to highlight a clear difference in the phenology between adults and immatures of the same taxon.

In species with a multimodal dispersal pattern, we found a majority of spiders inhabiting arable fields (agrobiont) with development synchronized with the arable-crop growing season (Samu & Szinetár 2002). Summer peaks in June and July are often the most important ones for these spiders

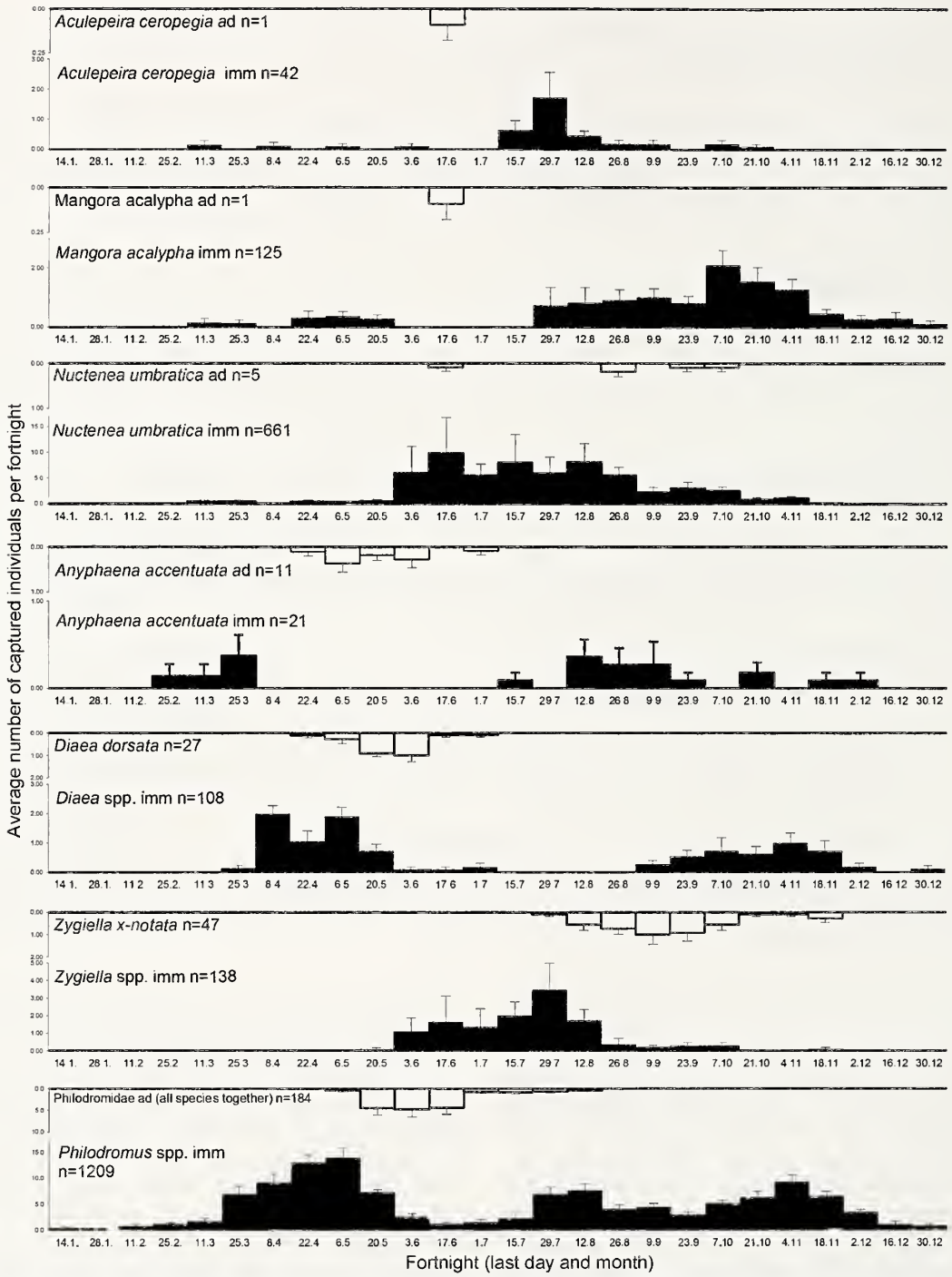

Figure 2.—Phenology (mean and standard deviation of the number of captured individuals per fortnight between 1994 and 2004) of taxa for which immatures and adults are identifiable. Upper panel: adults (white bars); lower panel: immatures (black bars).

because they coincide with their main reproductive season and also with the period of mowing and harvesting at the study site. Thorbek & Bilde (2004) found that agricultural management has a great impact on spider populations through direct mortality and triggering of dispersal. In contrast, almost all species with a unimodal pattern live in the upper strata of habitats with trees or bushes. These habitats are more stable than open ones. For these species, a short period for adult dispersal that is linked with reproduction appears to be a strategy sufficient to sustain populations.

The "multimodal" species are likely to have two generations of adults during the year (De Keer & Maelfait 1987, 1988; Topping & Sunderland 1998), with both of them ballooning. This bivoltinism is consistent with the observed average time between dispersal peaks (95 days) compared to the known development time of spiders (e.g., De Keer & Maelfait 1987, 1988). The autumn activity period is generally the shortest, probably because there are fewer hours with suitable conditions for ballooning (Thorbek et al. 2002). For species with a trimodal pattern, dispersals in late autumn (October–

November) and late winter (February–March) probably involve the same generation. The activity of the late winter period is very low and can only consist of overwintering adults. The *Oedothorax fuscus, Oedothorax apicatus* and *Araeoncus humilis* ballooning in late winter were almost exclusively females. Such a sexual bias has been noted for *Erigone atra* Blackwall 1833 (De Keer & Maelfait 1988), but we also caught males of this species (14 males and 20 females between 1 January and 18 February). Bell et al. (2005) suggested that the dispersal of fertilized females during these periods maximizes their reproductive success. It is interesting to note that these late autumn and late winter dispersals occurred at a height of 12.2 m, which contrasts with observations from Denmark where adults were rarely observed at such a height during this period (Toft 1995; Thorbek et al. 2002; Blandenier 2009).

The strategy of late and early dispersal may be triggered by human practices in field and crop habitats. In our study area, work in the fields is very intensive in October, greatly reduced in November, and absent until February, when work starts again at a low intensity. Therefore, dispersal in autumn allows spiders to reach new habitats where they overwinter (Thorbek & Bilde 2004). The maintenance of a low ballooning activity after this period allows the recolonization of suitable fields. This phenomenon is consistent with the observation of Gadgil (1971), who suggested that the best strategy for species inhabiting arable fields is to maintain a relatively high magnitude of dispersal at all density levels during various periods of the year.

Not surprisingly, almost all "unimodal" adult dispersers are stenochrones. The picture differed for juveniles of the same taxon for which analysis was possible; most showed a multimodal dispersal pattern.

Schaefer (1976) found immatures of the tree species *Anyphaena accentuata* (Walckenaer 1802) hibernating at ground level in the litter, and *Diaea* spp. and *Philodromus* spp. in the grass vegetation, which suggests a possible ontogenic change of stratum in the autumn dispersal peak. After winter, ballooning allows the recolonization of tree-shrub and herb layers. In contrast, Korenko & Pekár (2010) found that juveniles of the tree species *Anyphaena accentuata* and *Philodromus* spp. are winter-active on the bark of trees in the Czech Republic, and Hsieh & Linsenmair (2012) found *Anyphaena accentuata* hibernating in large numbers in the marcescent canopy of beeches in Germany.

Adult species that exhibit several dispersal peaks during the year are almost all eurychrones. Although adult eurychrones can be found year round, we observed that their aerial dispersal occurs at well-defined periods. *Erigone dentipalpis* is the only species with a quadrimodal pattern, and the time between summer peaks is accordingly small (52 days). It must be noted that interannual variability is very high for this species, and the observed quadrimodal distribution may partly result from accumulating 11 years of data. This question will be discussed in a further paper that analyzes the evolution of phenology for the seven most abundant species of this dataset (G. Blandenier et al. unpublished data). *Mermessus trilobatus,* an alien species in Switzerland (Wittenberg 2005) expanding its range in Europe (Eichenberger et al. 2009), is unique in that it shows a major autumn peak. This ability to reach new habitats late in the year may contribute to its colonization success.

When it was possible to identify adults and immatures of the same taxon, we observed a clear difference in ballooning pattern between life stages: immatures are generally bimodal, while adults are predominantly unimodal, with clear differences in the timing of activity periods (Fig. 2). This fact is particularly well illustrated in our study by immatures of *Diaea* spp. and adults of *Diaea dorsata* (Fabricius 1777), by immatures and adults of *Philodromus* spp., and by immatures and adults of *Anyphaena accentuata*, where the adults' peaks appear between those of the early instars. Judging from the number of individuals caught, ballooning appears to be more frequent in young instars. Rather than an ontogenic difference in ballooning propensity, this result may simply reflect higher population densities of immatures and the fact that they can balloon at higher altitudes (Bell et al., 2005). In this respect, most linyphiids captured were immatures (Blandenier 2009), but they are not considered here, since they could not be identified to the species level. Note that adults of large species (notably adults of large araneid *Nuctenea umbratica* (Clerck 1757) and *Aculepeira ceropegia* (Walckenaer 1802) were captured in the 12.2 m suction trap. The captured individuals were males, however, which are smaller than females (Foelix 2011).

Spiders have developed a wide array of life history strategies. Their colonization abilities are important and allow them to occupy a great variety of terrestrial habitats. The results of this study are consistent with the hypothesis that habitat perturbation triggers dispersal (Entling et al. 2011), in this way maximizing the survival of populations. The described diversity of ballooning phenological patterns may thus be a consequence of the various seasonal modifications and perturbations in their habitats. This hypothesis, however, overlaps with other explanations based on food availability (Mestre & Bonte 2012), competition, microclimate (Reynolds et al. 2007; Bonte et al. 2008), or small body size, which may differ in disturbed and undisturbed habitats.

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Pitfall trapping for surveying trapdoor spiders: the importance of timing, conditions and effort

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Abstract. Trapdoor spiders are challenging to sample using active searching methods because of their cryptic burrowing behavior. This poses problems for ecological studies and for gathering the data needed for conservation assessments. Pitfall trapping provides an alternative method that targets adult males as they wander from their burrows in search of females. Using pitfall trapping requires considerations of the timing of sampling within the year, the effects of environmental conditions on spider activity, and sampling effort required for a high probability of detecting species that are present at a sample site. To investigate these factors, pitfall-trapping surveys were conducted at several localities in Gauteng Province, South Africa. The results showed that trapdoor spider activity occurs in all seasons. Each species has a discrete period of activity ranging from a few weeks to several months in duration. Some species are active at different times of the year at different localities. Statistical analysis showed soil moisture as the only significant predictor of activity from amongst seven environmental variables, and the survey results indicate that trapdoor spiders are active under wet conditions following rainfall. Between two and seven nights of trapping are required for a high probability of detecting all species present and active at a site, using the trap design employed for this study with ten trap arrays per site. Trapping must be repeated at regular intervals throughout the year in order to obtain a near complete inventory of the species assemblage. The number of species collected ranged from two to eight per site, with most sites having six or eight species. Pitfall trapping yielded 1.2–3.0 times the number of species obtained by active searching at sites where both methods were employed. Guidelines for planning pitfall-trapping surveys of trapdoor spiders are presented and future research directions are discussed.

Keywords: Conservation, detection probability, Mygalomorphae, phenology, sampling

Sampling rare and elusive species is inherently challenging (Thompson 2004), which is problematic because these species are often most in need of conservation attention. Systematic conservation planning and biogeographical studies require sound species distribution data (Margules & Pressey 2000; Whittaker et al. 2005), and gathering such data requires effective sampling methods. Sampling rare and elusive species can require specialized or advanced field methods and sophisticated statistical tools. Small sample sizes are sometimes characteristic of such species, and low detection probabilities confound analysis (Lancia et al. 1994; Cunningham & Lindenmayer 2005; MacKenzie et al. 2005). Researchers should invest some effort in developing sampling methods that provide relatively high probabilities of detecting a species at a sample site if it occurs there, before undertaking further research for conservation purposes (McDonald 2004).

Trapdoor spiders are long-lived, slow reproducing, burrowing spiders with several species of conservation concern in different parts of the world (Clarke & Spier-Ashcroft 2003; Bond et al. 2006; Cooper et al. 2011; Engelbrecht & Prendini 2012). Many species construct burrows in the soil, which they cover with a lid that resembles the surrounding substrate. These burrows are often nearly impossible to detect with the naked eye. Increasing recognition of the conservation significance of these spiders and the resulting need for additional research requires effective sampling methods. The challenge is that their cryptic burrowing behavior makes them difficult to sample. Dippenaar-Schoeman (2002) and van Dam & Roberts (1917) described active searching methods for collecting trapdoor spiders, which include scanning the soil surface by eye, brushing away loose soil or surface debris, or seraping away the top few millimeters of soil with a spade to locate burrows. When burrows are located they are excavated to obtain the occupant. Pitfall trapping is a passive sampling method often employed in studies of ground-living, cursorial arthropods (Uetz & Unzicker 1976; Southwood & Henderson 2000; Woodcock 2005). Traps may be placed singly or in arrays or grids and may contain preservative or be used to obtain live specimens. Although juvenile and female trapdoor spiders are largely sedentary and seldom leave their burrows, adult males wander from their burrows in search of females (e.g., Main 1957; Coyle & Icenogle 1994; Dippenaar-Schoeman et al. 2006), which is when they fall into pitfall traps. This leads to male bias in pitfall trap samples, while active searching predominantly yields females and juveniles.

Trapdoor spiders are often collected in pitfall traps during general surveys of spider assemblages (e.g., Whitmore et al. 2001; Russell-Smith 2002; Warui et al. 2004; Dippenaar et al. 2008; Foord et al. 2008), but using this method to specifically inventory trapdoor spider assemblages requires consideration of timing of sampling and sampling effort. Timing of sampling is important because different species may be active at different times of the year and for varying durations, and activity may be affected by weather and other environmental conditions. Discrete periods of activity, or phenology, of adult male mygalomorphs have been demonstrated for two microstigmatid species occurring syntopically in KwaZulu-Natal, South Africa (Dippenaar-Schoeman et al. 2006); for antrodiaetids in North America (Coyle 1971; Coyle & Icenogle 1994) and are indicated in some taxonomic articles (e.g., Gallon 2002; Bond & Opell 2002; Wishart 2006, 2011). Coyle (1971) and Coyle & Icenogle (1994) noted that male antrodiaetid activity was also associated with rainy periods in North America, and Main (1957) stated that male idiopids wander after the first winter rains in southern Australia. Weather and other environmental conditions such as moon illuminanee affect

activity levels in many insect taxa and in turn influence trapping success (Williams 1940; Austin et al. 1976). To date no detailed studies of the influence of such factors on mygalomorph spiders have been published. Sampling effort is important because it has a significant influence on the probability of detecting a species at a sampling location if the species is present. As sampling effort increases, so the probability of detecting the species present increases. This has obvious implications for biodiversity surveys. The importance of including detection probability in ecological studies and monitoring programs has received significant attention (MaeKenzie et al. 2002; Kéry & Schmidt 2008; Guillera-Arroita et al. 2010).

The goals of the study presented here were as follows: 1) to investigate seasonal activity patterns, or phenology, of trapdoor spiders; 2) to assess the effects of weather and other environmental conditions on surface activity of trapdoor spiders; 3) to determine the sampling effort required to detect the species present in an assemblage with relative certainty and 4) to compare the effectiveness of active searching and pitfall trapping for inventorying species assemblages. The term 'trapdoor spider' is often applied loosely to several mygalomorph spider families, although some do not make trapdoors on their retreats. In this article it is used to refer to the families Ctenizidae, Cyrtaucheniidae, and Idiopidae in the South African context.

METHODS

Phenology and environmental conditions.—An initial survey was conducted at Roodeplaat Dam Nature Reserve (25°37′55″S, 28°21′25″E; 1235 masl), approximately 10 km NE of Pretoria, Gauteng Province, South Africa. Summers in northern Gauteng Province are warm and wet with temperatures reaching the mid-thirties Celsius during the day and with an average annual rainfall of around 650 mm (Schultz 1997). Winters are cool and dry with an average of 21 frost days per year (Schultz 1997). The survey was condueted in a homogenous habitat area approximately 15ha in size on a gentle, NW facing slope; red, sandy clay loam soil and sparse rock cover (< 1%). The vegetation is tall, open *Acacia caffra* woodland with bush clumps dominated by *Olea europaea* subsp. *africana*, *Euclea crispa*, *Ehretia rigida*, *Ziziphus mucronata* and *Protasparagus sauvedens*. The herb layer is dominated by the grasses *Eragrostis chloromelas* and *Cymbopogon excavatus*, with *Aloe greatheadii* var. *davyana* abundant. The reserve has a low abundance of game, and the study site is burned every few years as part of the management regime.

Pitfall trap cross arrays were used for the survey. Each trap array comprised a central trap with four arms of five traps, each radiating at right angles to each other from the central trap. Each individual trap consisted of a 13-cm diameter plant pot submerged in the ground with a 1-liter clear plastic container of the same diameter placed into it, with its rim flush with the soil surface. This design allowed the plastic tub to be removed for inspection during trapping periods without the hole in the ground collapsing. A plastic funnel 4 cm high and with an aperture of 6 cm was placed into the rim of the clear plastic container to prevent any captured specimens from climbing out. A mixed experimental design using 16 trap arrays was employed to determine if drift fences and trap density had an effect on the number of specimens captured in a trap array. Eight trap arrays had drift fences installed and eight did not, and eight had individual traps placed 1 m apart while the other eight had them placed 2 m apart. Drift fences were made of 150 mm wide, black polyethylene plastic strips stapled together and held in place between traps with 200-mm nails. The results of the trap design experiment are not reported here. The cross array design allowed for effective use of drift fences without the outer traps shielding the inner traps. The drift fences also made the trap arrays visible from a distanee so that they could be located easily and so that animals were less likely to trample them accidentally.

Trapping took place from early spring (19 September 2008) to late autumn (22 May 2009). Traps were opened every Monday afternoon and checked and closed between 06:00 and 07:00 the next morning. This procedure was repeated again on Thursdays, so that the traps were operated for two nights per week. No preservative was used in the traps. All live specimens found in the traps were marked with a small spot of writing correction ink on the posterior part of the thoracic region of the carapace and released in an attempt to determine population size using mark-recapture methods. Dead specimens, either drowned in rainwater that had accumulated overnight or killed by other occupants of the traps, were collected as vouchers for identification. Specimens were identified by comparing them with the original species descriptions and the type specimens of the species described from the Pretoria region, which are predominantly held in the Ditsong National Museum of Natural History (formerly the Transvaal Museum) in Pretoria.

Relationships between activity levels and environmental conditions were investigated for the four species with the largest sample sizes and for all species together. Counts of specimens per species were summed across all traps. For each species zero-counts outside of the period of activity were not included in the analysis. Count data from passive trapping combines the effects of varying population density and activity levels into a single measurement (Williams 1940; Curtis 1980; Southwood & Henderson 2000). Therefore, studies of one of these factors must control for the other (Briers et al. 2003; Høye & Forsehhammer 2008). Because surface activity was the factor of interest in this study, eounts were transformed to presence/absence of the species on each day that the traps were operated, to control for seasonal variation in abundanee of wandering males.

Logistic regression was used to analyze the relationship between activity and environmental conditions. Seven variables were included in the analysis. These were averages for temperature, humidity and wind speed; percentage moon illumination; evaporative demand of the atmosphere and relative soil moisture content on each night that the traps were open; and total rainfall over 72 h before the traps were checked. Hourly temperature, humidity, wind speed and rainfall data were obtained from a weather station situated approximately three kilometers from the sample site and used to caleulate nightly averages. Evaporative demand was calculated using a simplified Penman equation (Valiantzas 2006). Soil moisture was not measured, but was estimated for 15-cm soil depth (approximate average depth of spider burrows) using the point soil moisture balance model of

Table 1.—Localities where pitfall trapping surveys were conducted for trapdoor spiders in Gauteng Province with habitat descriptions and survey dates. Vegetation structural classifications follow Edwards (1983).

Survey site/Coordinates	Elevation (masl)	Annual Rainfall (mm)	Habitat	Survey season	No. of trap arrays
Boekenhoutskloof 25°34′42″S 28°28′58″E	1280	658	Short, moderately closed *Faurea saligna - Terminalia sericea* woodland on soft sand.	October 2010–June 2011	20
Onderstepoort Nature Reserve 25°36′31″S 28°07′07″E	1245	636	Low, moderately closed *Acacia nilotica* woodland on vertic clay soils.	October 2010–June 2011	20
Zwartkoppies 25°45′21″S 28°24′47″E	1350	681	Low, semi-open *Acacia karroo* woodland on dolorite derived, red structured clay soils.	October 2010–June 2011	20
Faerie Glen Nature Reserve 25°46′23″S 28°17′35″E	1360	696	Low, open mixed bushland on shale derived, red loamy clay soils. Significant weedy herb layer present.	October 2011–June 2012	10
Luipaardsvlei 26°13′33″S 27°43′23″E	1645	667	Low, semi-open grassland on red, sandy loam soil.	October 2011–June 2012	10
Uitzicht	1295	667	Low, semi-open mixed bushland on shale derived, red loamy clay soils.	October 2011–June 2012	10
Holgatfontein 26°25′21″S 28°34′19″E	1600	669	Short, closed grassland on a mosaic of sandy and melanic clay soils in a valley bottom.	October 2011–January 2012	10
Ezemvelo Nature Reserve (Lookout) 25°41′31″S 28°56′15″E	1370	681	Short, closed grassland on grey, gritty sandy loam soils.	February to April, 2012	10
Ezemvelo Nature Reserve (red sand) 25°42′36″S 28°56′28″E	1381	678	Tall, sub-continuous grassland on soft, red sand.	February to April, 2012	10

Rodriquez-Iturbe and Porporato (2004:32). Soil and vegetation parameter values needed for the model were obtained from Rodriquez-Iturbe and Porporato (2004), Scholes and Walker (1993) and the Agricultural Research Council's Institute for Soil, Climate and Water. All seven environmental variables were included simultaneously in the analysis, without interactions, to identify any significant predictors of activity.

Sampling effort.—Following the study at Roodeplaat Dam Nature Reserve, pitfall trapping surveys were undertaken at several other localities in Gauteng Province representing a range of habitat types (Table 1, Fig. 1). A trap design similar to that described above was used. Trap arrays of 21 traps each were used, with traps 1 m apart and with drift fences installed. Traps at each site were operated for one to five days after rainfall events, with sampling events between two weeks to a month apart, from October to the following May. The number of adult males per species was recorded per trap array. Voucher specimens were collected for all species at all sites.

To determine detection probabilities relative to sampling effort, the data were first aggregated to obtain the number of times a species was caught in a trap array (successes) and the total number of trap arrays operated across all trapping events where that species was collected (trials) at a site. The ratio of successes to trials gives an estimate of the average probability that the species will be caught in a trap array in one trapping event, given that the species is active. A 95% confidence interval estimate for this proportion was obtained using the Wilson interval estimator, which provides robust estimates for proportions of small samples and values near zero or one (Agresti & Coull 1998). These intervals were used to calculate the number of trap array events needed to detect the species for a range of detection probability values using the geometric distribution. Results for species that were collected only once at a site were not included because they gave estimates of sampling effort that were too imprecise to be informative.

Species accumulation curves were plotted to assess the completeness of the species inventory at each site. Rarefaction with 1000 iterations per sample size was used to produce smooth curves (Gotelli & Colwell 2001). Curves were not produced for sites that were not sampled for an entire summer season, or for Boekenhoutskloof where only two trapdoor spider species were collected despite a sampling effort similar to other sites. The habitat at this site is a seasonally inundated bottom-slope with a shallow hard plinthic soil horizon. The periodic inundation that results from the shallow plinthic layer likely accounts for the low number of species collected there.

Active searching was also undertaken at the sampling sites at Onderstepoort Nature Reserve, Zwartkoppies, Luipaardsvlei and Faerie Glen Nature Reserve. The primary purpose was to obtain females of the species collected during pitfall trapping at these sites. Soil scraping was employed to locate burrows. Experience showed that soil scraping was superior to brushing the surface or scanning the soil surface by eye. Combined effort was not standardized and varied between the sites from approximately 12 h at Luipaardsvlei to over 30 h at Faerie Glen Nature Reserve and Zwartkoppies, and took place at different times of year. Only a subjective comparison of the two methods is possible, and the results are discussed accordingly. All mygalomorph families were included in the comparison.

A map of the study area showing sampling localities was prepared with ArcGIS Version 9.3 (Fig. 1). All analyses were

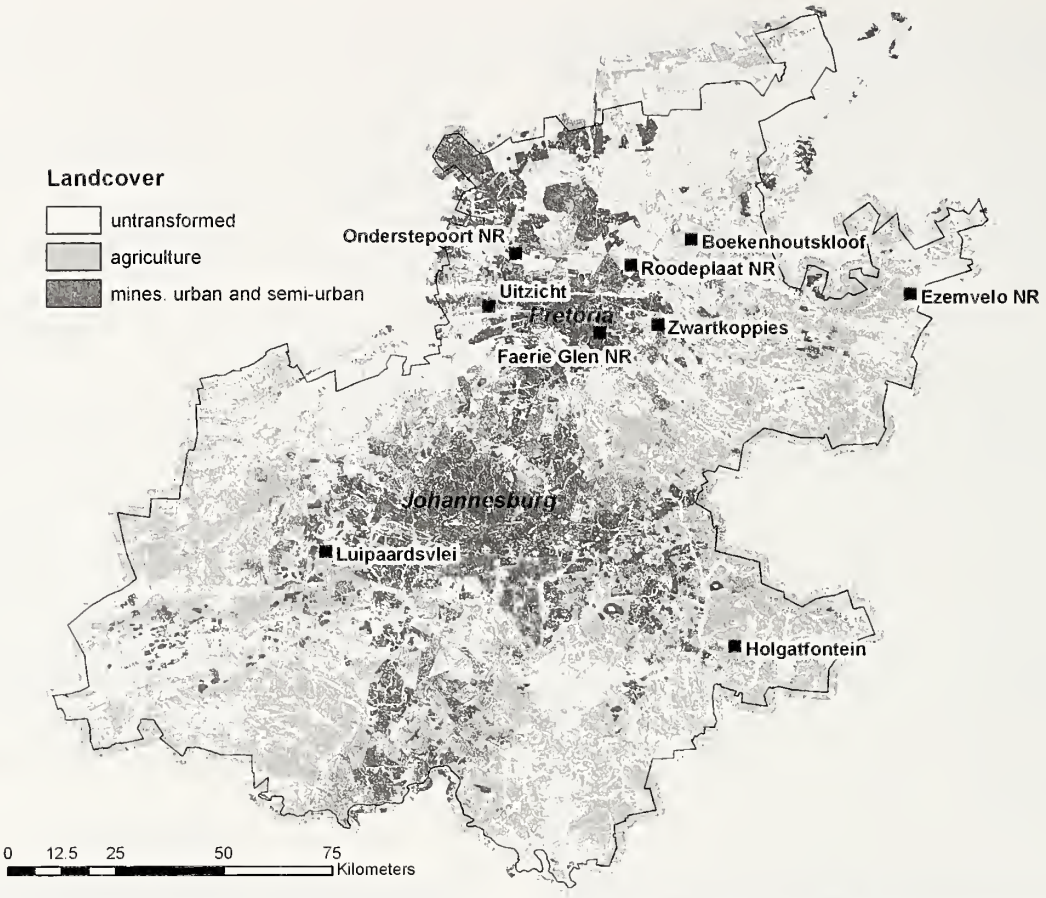

Figure 1.—Map of pitfall trap surveys localities for trapdoor spiders in Gauteng Province, South Africa, showing major urban and agricultural transformation. Landcover derived from GeoTerraImage (2009).

conducted with R Version 2.14.1 (R Development Core Team 2011).

RESULTS AND DISCUSSION

Phenology and environmental conditions.—A total of 220 trapdoor spider specimens, comprising eight species, were collected during the survey at Roodeplaat Dam Nature Reserve. 180 of these were marked and released, but none were recaptured. *Stasimopus hewitti* Engelbrecht & Prendini 2012 was the most abundant species with 135 specimens collected, followed by *Ancylotrypa brevipalpis* (Hewitt 1916) with 53 specimens. *Ancylotrypa rufescens* (Hewitt 1916), *Galeosoma hirsutum* Hewitt 1916, *Gorgyrella schreineri minor* (Hewitt 1916) and *Idiops pretoriae* (Poeock 1898) were the rarest species at the site, with two to four specimens collected for each species. *Ancylotrypa pretoriae* (Hewitt 1913) and *Segregara transvaalensis* (Hewitt 1913) were more abundant, with 10 and 11 specimens collected respectively. In addition, the theraphosid species *Brachionopus pretoriae* Purcell 1904 was also collected in the pitfall traps, but only females were trapped. Another theraphosid, *Augacephalus junodi* (Simon 1904), was observed at the site but was never trapped.

Males of *Ancylotrypa brevipalpis*, *Galeosoma hirsutum* and *Idiops pretoriae* were active during October and November (Fig. 2). *Galeosoma hirsutum* and *I. pretoriae* had a relatively short activity period of two to three weeks, while *A. brevipalpis* was active for almost two months. *Stasimopus hewitti* was active for almost three months over the midsummer period of November to February, and *Gorgyrella schreineri minor* was active over three weeks in January. *Ancylotrypa pretoriae* was active from midsummer to early autumn (January to March), *A. rufescens* in late autumn (May) and *Segregara transvaalensis* in autumn (March to May).

Ancylotrypa brevipalpis and *Stasimopus hewitti* both showed a bell-shaped change in abundance through their activity period, with more specimens trapped in the middle of the activity period. A significantly larger than average number of specimens of each of these species was collected on a single occasion. For *S. hewitti* this occurred in early January, at approximately the middle of the activity period, with 50 specimens trapped, while for *A. brevipalpis* it occurred in early December, toward the end of the activity period, with 23 specimens trapped. The trimmed mean number of specimens trapped per day for each species was six for *S. hewitti* and three for *A. brevipalpis*.

Logistic regression of activity against environmental conditions identified no significant predictors of activity for any of the species analyzed individually, but showed soil moisture to be a significant predictor of activity when all species were analyzed together ($P = 0.004$). Plots of activity and rainfall show that male activity tended to occur within rainy periods (Fig. 2). It was also clearly apparent while doing the fieldwork that male trapdoor spiders would only be collected for a few days following significant rainfall events when the soil was

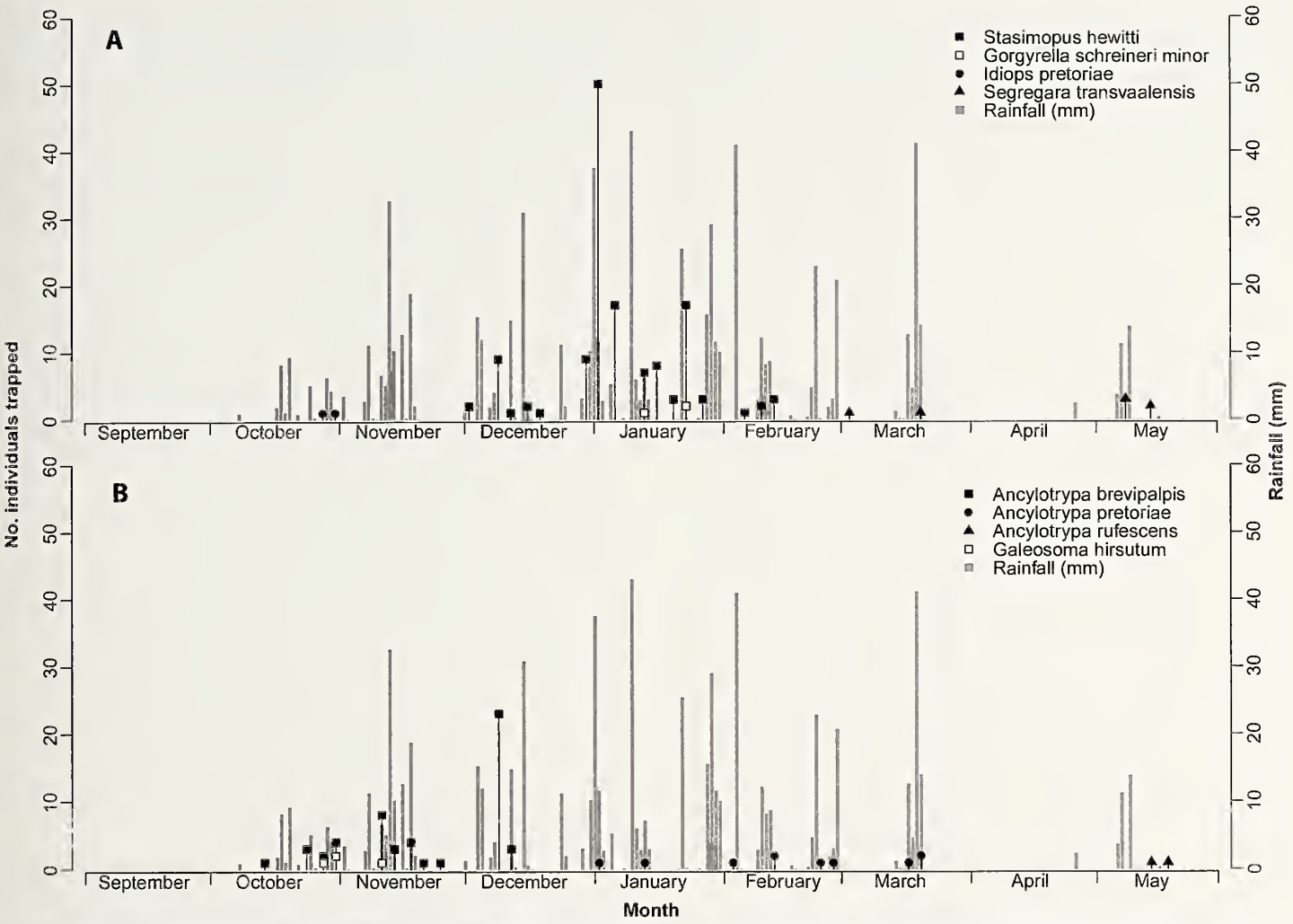

Figure 2.—Spike plots showing phenology of adult male trapdoor spiders, measured as number of individuals captured, at Roodeplaat Dam Nature Reserve, Gauteng Province, South Africa, as assessed during a pitfall trapping survey from September 2008 to May 2009.

visibly wet and there was significant dew formation. Plots of the raw data showed that activity was more common when humidity was high and when wind speed and evaporative demand were low. However, no activity also occurred under the same conditions, suggesting that measured environmental variables interact in a complex manner to influence surface activity in male trapdoor spiders. Low sample sizes, such as those obtained in this study, and which are likely to be characteristic of studies of mygalomorph spiders, are a limitation to identifying the nature of such complex relationships.

Subsequent sampling at other sites in the province confirmed the discrete periodicity of male activity and its dependence on the wet weather conditions observed at Roodeplaat. There appears to be some degree of seasonally-based correlation in activity between different species so that particular subsets of the total species assemblage are active at different times of the year. Broadly speaking these activity periods are October to November (early summer), November to February (mid-summer), February to April (autumn), and April to June (winter). Species active in the early summer period tend to have short activity periods of a few weeks. Those that are active in mid-summer and autumn are active for longer periods. The observation of activity by some species in the winter months is unusual for mygalomorphs. An *Ancylotrypa* species was collected after two rainfall events in late May and mid-June at Boekenhoutskloof, and photographic evidence of male *Stasimopus robertsi* Hewitt 1910 active in June at Faerie Glen Nature Reserve, also following rainfall, was provided to the author by a member of the public. Due the rarity of winter rainfall events during the study period the data were insufficient to make general conclusions about winter activity patterns. In general, more species were active in the early summer and autumn periods than in mid-summer or winter. Activity peaked toward the end of April in the autumn period. Within the different families most idiopid species were active in the early summer and autumn periods, *Stasimopus* were never recorded in early summer, and cyrtaucheniids were active in all periods.

The general patterns in periodicity of activity described above were not without exceptions. Some species were found to be active at different times of the year at different localities. An example is *Segregara transvaalensis*, which was active from March to May at most localities, but was active in October and

November at Onderstepoort and a site sampled near Hekpoort. Another example is *Ancylotrypa nigriceps* (Purcell 1902), which was active from midsummer to autumn at most localities but was only active for a short period in spring at Zwartkoppies. Single or small numbers of specimens were occasionally trapped outside of their usual activity periods. *Segregara transvaalensis* was collected in early September at Roodeplaat in the spring following the initial survey there. Single specimens of an undescribed *Stasimopus* Simon 1892 species and *S. transvaalensis* were collected in August at Zwartkoppies, and a single specimen of an undescribed *Segregara* Tucker 1917 species was collected at Luipaardsvlei in early summer where that species was found to be commonly active in autumn.

These surveys suggest that moisture is important for activity in atypids, ctenizids, cyrtaucheniids and idiopids, which were generally only collected under wet conditions following rainfall. Theraphosids and nemesiids appeared to be more tolerant of drier conditions. The emergences of adult male trapdoor spiders following rainfall have also been observed in other parts of South Africa and in East Africa (Neethling & Haddad 2011; J. Leeming pers. comm.; P. Hawkes pers. comm.; I. Englebrecht pers. observ.). Some species that are active later in the season may also delay activity until several days after rainfall. *Stasimopus robertsi* males at Faerie Glen Nature Reserve only started wandering four days following a rainfall event in late April. The soil was still damp, and other trapdoor spider species were still active at the same time. The photographic record of this species provided to the author from the same locality was also taken several days after rainfall in June the year before. These observations, together with those of Coyle (1971), Coyle & Icenogle (1994) and Main (1957), suggest a general pattern of rainfall-related activity for several mygalomorph families.

Sampling effort.—The average sampling effort required for a high probability of detecting all species present and active at a site ranged from two to seven nights of trapping using 10 trap arrays (Table 2). This seems to be a reasonable estimate for optimal sampling effort, given the experience gained during the study. Importantly, this must be interpreted as the sampling effort needed under suitable environmental conditions for spider activity and must be repeated at appropriate intervals throughout the year in order to obtain an inventory of the species present at a site. The large upper estimates of effort needed for some species indicated in Table 2 should be interpreted conservatively. These arise because the Wilson estimator produces less precise estimates for capture probability when the proportion of trials to successes is low (Agresti & Coull 1998).

The species accumulation curves for Roodeplaat, Onderstepoort and Zwartkoppies are asymptotic, suggesting complete or near complete inventories for these sites, while those for Uitzieht, Faerie Glen and Luipaardsvlei are more linear (Fig 3). Rather than indicating incomplete inventories, the curves for Uitzicht, Faerie Glen and Luipaardsvlei result from a change in the sampling strategy employed. These sites were sampled about half as often as the others, with half the number of traps. Knowledge gained from the the earlier surveys was used to determine the timing and frequency of sampling needed to detect all species present with minimal sampling effort. An ideal sampling strategy with this objective should produce a linear species accumulation curve up to the total number of species present at the site. Departures from linearity would indicate sampling inefficiency. Such linear accumulation curves are not useful for evaluating inventory completeness, but given that the numbers of species collected was comparable to those obtained at other sites, the inventories are also likely to be complete or near complete.

The values in Table 2 and Fig. 3 can be used in planning future pitfall trapping surveys for trapdoor spiders by determining how much sampling effort is required for a certain pre-specified probability of detecting the species present, or for interpreting the results of surveys where sampling effort was limited by resource constraints (McArdle 1990). Planning should make provision for failed trapping events, such as when traps are flooded by heavy rainfall or when rainfall is insufficient to trigger spider activity. Studies should be planned according to clearly defined objectives, which can in turn be used to specify sampling effort. Yoccoz et al. (2001), MacKenzie & Royle (2005) and Guillera-Arroita et al. (2010) provide guidelines for planning surveys with specific consideration of detection probability. In general, studies which aim to determine the presence of a single species at a single site, such as an EIA aiming to determine if a threatened species is present at a potential development site, should aim for the highest detection probability possible given the available resources. Occupancy studies, which aim to determine the proportion of sites where a species occurs, or studies that investigate species–environment relationships, can still provide valid results with lower detection probabilities as long as the appropriate statistical models are used to analyze the results (MacKenzie et al. 2002).

In comparing the relative efficacy of pitfall trapping and active searching, pitfall trapping yielded more species than active searching in all cases. At Zwartkoppies, pitfall trapping yielded seven species. Active searching failed to locate one of these and yielded only a single subadult specimen of another. At Onderstepoort pitfall trapping yielded six species and active searching yielded three, and at Luipaardsvlei pitfall trapping yielded six species while active searching yielded two. At Faerie Glen Nature Reserve pitfall trapping yielded eight species, while active searching yielded four. Total pitfall trapping yielded 1.2–3 times the number of species obtained by active searching at sites where both methods were employed. Active searching did not yield species that were not also caught in pitfall traps at any of the sites where both methods were employed. These results indicate that short-term active sampling efforts are unlikely to produce a complete inventory of a trapdoor spider speeies assemblage.

As expected both methods resulted in highly sexually biased samples, with active searching yielding primarily adult females and juveniles while pitfall trapping predominantly yielded adult males. Active searching was also more effective in habitats with harder, more clayey soils than in habitats with softer, sandier soils. This was probably due to the stronger soil structure of clayey soils, where burrows were less likely to collapse when the surface soil layer was removed. Dense vegetation covering the soil surface also made active searching more difficult, as burrows were presumably covered or collapsed in the proeess of removing the vegetation. Both methods also have their drawbacks. Active searching is labor

Table 2.—Estimated detection probabilities and sampling effort for selected trapdoor spider species in Gauteng Province, South Africa. x: Number of successes, i.e., specimens collected in a trap array. x: number of captures, n: Number of trials, i.e., total number of trap arrays operated for trapping events when the species was?collected.

Family/Species	Locality	x	n	Estimated capture probability per trap array event.	Number of trap arrays required for 90% detection probability.
Atypidae *Calommata transvaalica*	Zwartkoppies	9	60	0.081–0.261	8–28
Ctenzidae *Stasimopus hewitti*	Roodeplaat	56	256	0.173–0.273	8–13
	Faerie Glen NR	10	38	0.150–0.420	5–15
	Uitzicht	3	20	0.052–0.360	6–43
Stasimopus sp. 1	Holgatfontein	2	18	0.031–0.328	6–74
Stasimopus sp. 2	Luipaardsvlei	6	10	0.313–0.832	2–7
Stasimopus sp. 3	Onderstepoort NR	9	58	0.084–0.269	8–27
Stasimopus sp. 4	Zwartkoppies	2	20	0.028–0.301	7–82
Cyrtaucheniidae *Ancylotrypa*	Roodeplaat	28	176	0.112–0.220	10–20
brevipalpis	Luipaardsvlei	8	8	0.676–1.000	1–3
Ancylotrypa nigriceps	Holgatfontein	3	18	0.058–0.392	5–39
	Luipaardsvlei	2	10	0.057–0.510	4–40
	Zwartkoppies	5	70	0.031–0.157	14–74
Ancylotrypa pretoriae	Onderstepoort NR	15	54	0.176–0.409	5–12
	Roodeplaat	11	160	0.039–0.119	19–59
	Faerie Glen NR	15	20	0.531–0.888	2–4
	Zwartkoppies	5	20	0.112–0.469	4–20
Ancylotrypa rufescens	Boekenhoutskloof	5	40	0.055–0.261	8–42
	Ezemvelo NR (Lookout)	11	20	0.342–0.742	2–6
	Ezemvelo NR (red sand)	13	20	0.433–0.819	2–5
Idiopidae *Galeosoma hirsutum*	Roodeplaat	3	48	0.022–0.168	13–107
	Zwartkoppies	23	100	0.158–0.322	6–14
Galeosoma robertsi	Faerie Glen NR	4	8	0.215–0.785	2–10
	Onderstepoort NR	36	50	0.583–0.825	2–3
	Uitzicht	10	40	0.142–0.402	5–16
Gorgyrella schreineri minor	Ezemvelo NR (Lookout)	4	10	0.168–0.687	2–13
	Roodeplaat	2	32	0.017–0.202	11–132
Idiops fryi	Ezemvelo NR (Lookout)	3	10	0.108–0.603	3–21
Idiops nigropilosus	Holgatfontein	11	25	0.267–0.629	3–8
Idiops pretoriae	Roodeplaat	2	32	0.017–0.202	11–132
	Faerie Glen NR	3	8	0.137–0.694	2–16
	Zwartkoppies	20	100	0.133–0.289	7–17
Idiops sp.	Luipaardsvlei	5	10	0.237–0.763	2–9
Segregara sp.	Luipaardsvlei	7	18	0.203–0.614	3–11
Segregara transvaalensis	Roodeplaat	10	80	0.069–0.215	10–33
	Ezemvelo NR (Lookout)	8	10	0.490–0.943	1–4
	Ezemvelo NR (red sand)	14	20	0.481–0.855	2–4
	Faerie Glen NR	3	20	0.052–0.360	6–43
	Onderstepoort NR	11	50	0.126–0.352	6–17
	Uitzicht	2	10	0.057–0.510	4–40

intensive and often yields small numbers of specimens, which can be frustrating for the collector. Pitfall traps can get flooded by rain or damaged by animals. Where preservative is used, large quantities of unwanted by-catch, including small vertebrates, are killed and the preservative, which is usually toxic, may pose a threat to curious wildlife and livestock. The pitfall trapping method used for the surveys conducted in this study, while still labor intensive, was found to be preferable to active searching as it produced larger numbers of specimens and more species, and it avoided the problems associated with trapping using preservatives. Adult male spiders are also more useful for taxonomic and identification purposes than females, which further supports the use of pitfall trapping as the primary sampling method in trapdoor spider surveys. Although a more rigorous comparison of the relative efficacy of these two methods is needed, pitfall trapping appears to be the better option if time and resources are available.

Based on the results of the surveys described in this article the following principles should guide future surveys of trapdoor spider communities. Pitfall trapping is more effective than active searching when employed throughout the period of the year that adult male trapdoor spiders are active. Pitfall trapping should be used preferentially where long-term surveys are possible. Two to seven nights of trapping with ten trap arrays installed will give a high probability of detecting all species present and active at the time of trapping. To obtain complete or near complete species inventories, these sampling events should be repeated at regular intervals throughout the year, including the winter months, as different species are active at different times of the year. As several

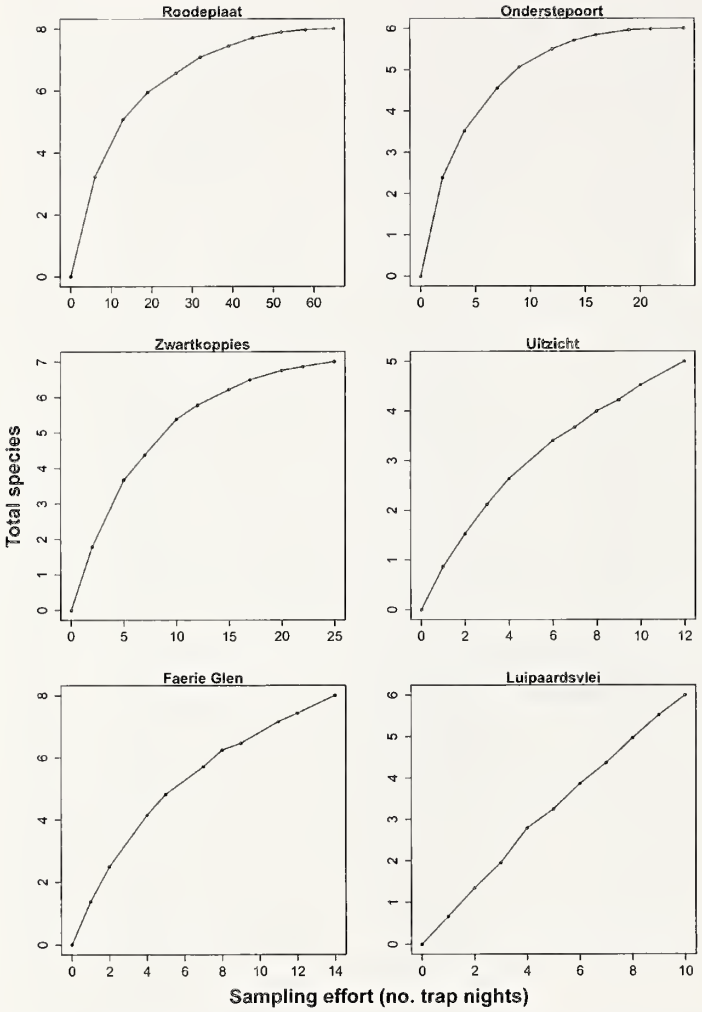

Figure 3.—Species accumulation curves for six localities surveyed for trapdoor spiders in Gauteng Province. Curves were produced by rarefaction with 1000 iterations per sample size. See text for discussion of inventory completeness.

species show relatively short activity periods of less than a month, sampling should take place approximately every two to three weeks, depending on suitable conditions. Sampling in early summer (mid-October to early November) and in autumn (late April and early May) are especially important, as more species are active during these periods than at other times of the year. Sampling should take place following rainfall events, as most trapdoor spider species are active under wet conditions, and should continue for several days so that species that delay their activity following rainfall are also detected. Sampling under dry conditions is generally unsuccessful for trapdoor spiders.

This study has provided guidelines for future surveys of trapdoor spiders in Gauteng Province. Resolving the current inadequacies in trapdoor spider systematics (Engelbrecht & Prendini 2011), and ultimately gathering the data needed for conservation assessments, will require a substantial amount of survey work in the future. This should take place in a planned and structured way using the principles described above so as to maximize the return on the resources invested in surveys. Further research on the surface activity of male trapdoor spiders should include more detailed studies of weather-related activity that specifically aims to test the hypothesis that rainfall or moisture is the principal driver. Maximizing sample size should be a primary goal in such studies. Data analysis should consider interactions between variables and alternative methods of controlling for seasonal variation in abundance in order to make maximal use of raw count data. Before commencing major survey efforts researchers should invest in an effort to determine the times of the year that trapdoor spiders are active, particularly where climatic conditions differ from those in Gauteng Province. Finally, more accurate estimates of species-detection probabilities will be critical for planning surveys and analyzing survey results.

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The impact of structural and landscape features of set-asides on the spiders (Araneae) of the herb layer

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Abstract. We investigated the effects of area, age, vegetation structure and landscape features of set-asides on the spiders of the herb layer. We caught the spiders using a semi-quantitative sweep netting of the herb stratum in 160 sampling plots at 32 set-asides in the northeastern lowland of Brandenburg, Germany, from May through August 2001. We analyzed the data using multiple linear regression. The results revealed the following. 1) Vegetation height was the most influential factor increasing the number of species and individuals of particular araneid species at the set-asides. 2) Vegetation cover had no significant effect on the total number of species, but did affect the abundance of particular araneid and linyphiid species. 3) Time since the set-aside establishment and time since last management had no significant influence on the number of species, the number of individuals of particular species, the number of individuals of the ecological group "preferred habitat type." 4) Different types of vegetation structures were used by spider families and araneid species in different ways; the abundance of some araneids benefited from high (dense or sparse) vegetation, whereas linyphiids only benefited from dense vegetation cover.

Keywords: Arable field, landscape ecology, landscape matrix, vegetation structure

Recently the impact of structural, temporal, and landscape factors on the species composition and, in particular, species richness of the spiders of the herb layer in agrarian landscapes has been intensively discussed. Uetz (1991), Gibson et al. (1992a) and Wise (1993) hypothesized that herb-dwelling spiders would have relatively predictable assemblages based on habitat structure. Furthermore, Gibson et al. (1992b) expected the spiders of the herb layer to depend directly on the struetural complexity of the vegetation and thus respond to variation in the plant structure on a narrow, local level. These suggestions were supported by several investigations on the influence of vegetation structure on species composition and abundance of herb-dwelling spiders (Scheidler 1990; Borges & Brown 2001; Ysnel & Canard 2000). Rypstra et al. (1999) emphasized the significance of vegetation configuration for the web-attachment points of the spiders of the herb layer. In addition, Schmidt et al. (2003, 2005, 2008) stressed the particular influence of high vertical vegetation structures in arable fields on the abundance of web-building spiders. Most of these reports described the positive effects of diverse vegetation structure in terms of the benefits of the habitat for spiders as pest predators and the promotion of species diversity i.e., the eontribution to nature conservation in the agrarian landscape (Schmidt & Tscharntke 2005a, b).

It is increasingly recognized that, beside the vegetation structure and the age of set-asides themselves, the distribution and size of the surrounding habitat patches can strongly influence the local diversity and abundance of organisms (Dauber et al. 2003; Duelli & Obrist 2003; Jeanneret et al. 2003; van Buskirk & Willi 2004; Tscharntke et al. 2011). Bell et al. (1998), Perner & Malt (2003) and Frank & Reichert (2004) found that species richness and the number of individuals of both ground and herb-dwelling spiders increased with the age of set-asides (abandoned farmland) and field edges, respectively.

Numerous recent studies have examined the impact of the surrounding landscape matrix on the ground-dwelling spiders in arable fields (Schmidt & Tscharntke 2005a, b; Schmidt et al. 2003, 2005, 2008; Öberg et al. 2008). Schmidt-Entling & Döbeli (2009) and Haaland et al. (2011) found that sown wildflower strips enhance the species composition and species richness of spiders in arable fields and along the margins of the fields. However, very few investigations have considered the simultaneous impacts of vegetation structure, spatial and temporal properties of the local habitats and the composition of the surrounding landscape matrix on herb-dwelling spiders (however, see Schmidt-Entling & Döbeli 2009).

In our study we hypothesized that the number of species and individuals of herb-dwelling spiders, the response variables, would benefit from vegetation height and cover of the herb layer at the set-asides. We assumed a positive relation between area and time since establishing the set-asides and the response variables. Further, we hypothesized that the distance of different habitat types would have an increasing or diminishing effect on the number of individuals of particular herb dwelling species depending on their preferred habitat as well as on the number of individuals of the ecological group "dry open habitats".

To check our hypotheses, we simultaneously tested the impacts of spatial and temporal factors on a local scale and the landscape features on herb-dwelling spiders to find the most influential predictors that determine species richness and abundanee. Specifically, we tested the impact of the following variables: a) the area of the set-asides; b) the age of the plots and the time since they were last managed; c) the height and cover of the vegetation and d) the distance from the transect to the nearest habitat other than a set-aside on the overall number of species, the number of individuals of particular species and the ecological group "dry open habitats".

METHODS

Study Sites.—The area of investigation was located on the north-eastern lowland of Germany. The study was conducted

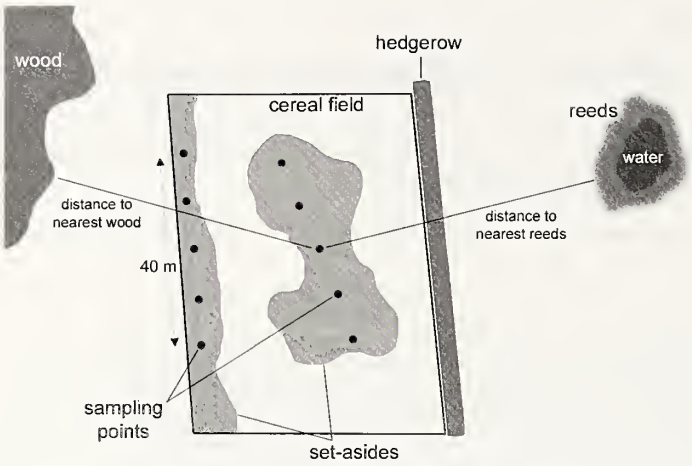

Figure 1.—Sampling design (not scaled) for both the web-spider catches and vegetation surveys. See the text for further explanation.

in northern and southern Uckermark and on the Lebus plate in the Müncheberg area. The young moraine landscape in the Uckermark region is characterized by heterogeneous site conditions. The soils range from very sandy to loamy or partly fen. The fields often contain steep slopes bordered by flat areas or a heterogeneous mix of wet and dry spots. In contrast, the soils of the Müncheberg area are predominantly sandy. In all of these regions, the annual precipitation ranges between 357 and 793 mm/year, the average temperature is 8.8°C [Meteorological station Angermünde and Müncheberg, median values and annual total, respectively, 1973–2002; Federal Ministry of Transport, Building and Urban Development (Deutscher Wetterdienst), 2011, unpublished data].

The farms range from 1,000 to 2,000 ha in area. The average size of the investigated plots (northern Uckermark, 18; southern Uckermark, 6; Müncheberg area, 8) was 2.35 ha ± 2.01 ha. The set-asides were 714 to 3,644 days old; the time since they were last managed up to the last sampling date ranged from 29 to 3,637 days. The management aetivities included mulching and, less frequently, grubbing in the springtime (May or June) and autumn (September or October) to suppress weeds such as thistles. A number of the set-asides were already established in the mid-1990s when the European Union granted subsidies to farmers to set aside parts of their fields to enhance the species richness of the flora and fauna in the agrarian landscape (for details, see Berger et al. 2003, 2006). The detailed data on the spatial and temporal variables are given in Table A1 (see Appendix).

Sampling.—The spiders of the herb layer were caught at a total of 160 sampling points on 32 set-asides within and adjacent to cereal fields. Each of the set-asides was sampled along a 40 m transect situated at the center of the plot. The spiders were caught at five sampling points arranged in a straight line at 10 m intervals (see Fig.1).

Each plot was sampled four times in 2001, during the third week of May, June, July and August, using a semi-quantitative sweep netting procedure (Witsack 1975). The spider collections were performed for 10 minutes at each of the five sampling points within the plot; thus, the total sampling time was 50 minutes on each transect. We surveyed the vegetation structure (measurement of the vegetation height and visually estimation of the percentage vegetation cover) of the herb-, and grass-layer of 10 1x1-m plots at a distance of 1m on both sides of each sampling point (see Figure 1) at each sampling date according to a method of Dierschke (1994). On a landscape scale, we calculated the nearest distances from the adjacent non-set-aside habitat types (arable fields, waters, reeds and woods) to each of the set-aside plots using Geographic Information Systems (GIS) maps (see Figure 1).

Most of the adult spiders were identified in the field with a magnifying glass (10 ×) and then released. *Dictyna arundinacea* (Linnaeus 1758), *Metellina segmentata* (Clerck 1757), *Tetragnatha extensa* (Linnaeus 1758), and all juveniles, linyphiid and theridiid spiders were transferred to jars containing 70% ethanol, transported to the laboratory and identified using Heimer & Nentwig, (1991), Roberts (1985, 1987, 1995), and Wiehle (1956, 1960). The nomenclature follows Platnick (2011). Those spiders that were not identified to the species level, sueh as the juveniles of Salticidae and Thomisidae, are not considered in this report because our statistical analyses were based exclusively on the species data.

Ecological groups.—The term "preferred habitat type" used in this report acts as an ecological group that represents the local distribution of a spider species in the landscape of the federal country of Brandenburg. Each species found in this federal country was assigned to one of 19 defined habitat types or to "unknown (?)" if an unambiguous allocation was not possible (Platen et al. 1991, 1999). The preferred habitat types for each species caught in this investigation are listed in Table 1. The preferred habitat types "fallowland", "dry grassland", and "heather" that are similar in low shading and low moisture were combined to "dry open habitats" and were tested as a combination.

Statistical analyses.—First, the predictor variables vegetation height and vegetation cover per sampling point and each sampling date were averaged for each set-aside. A multiple linear regression (Sokal & Rohlf 1995) was performed to test the hypotheses formulated above. All of the predictors were tested for multiple colinearity. The tolerance and variance inflation factor (VIF) were also calculated. Moreover, the data were tested for autocorrelation with Durbin-Watson statistics. The corresponding procedures are implemented in the program SPSS. The test of the predictor variables for multiple colinearity showed a tolerance between 0.26 and 0.73. The variance inflation factors (VIFs) ranged between 1.4 and 3.8. In general, the borders to reject independence are <0.25 for the tolerance and >5.0 for the VIF, respectively (Urban & Mayerl 2006). The results of the Durbin-Watson statistics ranged from 1.27 to 2.30 (see Table 2). For N=32 and k=10 the lower limit is d_L=0.59, the upper limit d_U=2.13 where N is the number of cases and k the number of predictors (Savin & White 1977).

Each of the response variables, i.e. species, the family Linyphiidae and the preferred habitat type were tested independently for significanee in a separate model. All of the variables tested were included in each of the models simultaneously. Before the analyses, the number of individuals of each species was summed over the four sampling periods of the investigation. Only those response variables that showed a significant result for at least one predictor are displayed in Table 2, including the overall number of species, the numbers of individuals of particular araneid and linyphiid

Table 1.—Total numbers of adult and juvenile spider species caught at the set-asides in northeast Brandenburg during four months, with modified habitat preferences according to Platen et al. (1991, 1999).

Species	Number of individuals		Preferred habitat
	Adults	Juveniles	
Aculepeira ceropegia (Walckenaer 1802)	1	277	Agricultural field
Agalanatea redii (Scopoli 1763)	87	0	Fallowland
Araneus diadematus Clerck 1757	25	3	Dry forest
Araneus quadratus Clerck 1757	203	58	Wet grassland
Argiope bruennichi (Scopoli 1772)	91	80	Fallowland
Cyclosa oculata (Walckenaer 1802)	2	8	Fallowland
Cheiracanthium erraticum (Walckenaer 1802)	2	0	Dry grassland
Dictyna arundinacea (Linnaeus 1758)	62	21	Fallowland
Enoplognatha ovata (Clerck 1757)	39	3	Fallowland
Erigone atra Blackwall 1833	2	0	Agricultural field
Floronia bucculenta (Clerck 1757)	71	0	Wet forest
Larinioides cornutus (Clerck 1757)	12	313	Reeds
Larinioides patagiatus (Clerck 1757)	4	0	Dry forest edge
Linyphia triangularis (Clerck 1757)	83	5	Dry forest
Mangora acalypha (Walckenaer 1802)	42	1255	Agricultural field
Metellina segmentata (Clerck 1757)	4	1	Moist forest
Microlinyphia pusilla (Sundevall 1830)	1712	1127	Fallowland
Neoscona adianta (Walckenaer 1802)	1	3	Heather
Phylloneta impressa (L. Koch 1881)	628	936	Fallowland
Pisaura mirabilis (Clerck 1757)	7	36	Fallowland
Selimus vittatus (C.L. Koch 1836)	2	0	Dry forest
Tetragnatha extensa (Linnaeus 1758)	9	92	Reeds
Tibellus oblongus (Walckenaer 1802)	1	15	Fallowland

species, the linyphiid family as a whole and the preferred habitat type "dry open habitats". Each analysis was calculated separately for the abundance of adult and juvenile spiders. As the results for adults and juveniles showed no significant differences, the analyses were only displayed for the developmental stages combined together. Before the analyses, all response variables were logarithmically transformed (log(x+1)) to normalize the distribution and homogenize the variance. The distances from the set-aside plots to different adjacent habitat types up to 1 km from each set-aside were calculated using GIS maps (Ministerium für Umwelt, Naturschutz und Raumordnung 1995, Scale, 1:10,000). The computer programs used were ArcView version 3.3, (ESRI Inc., Redlands, CA, USA), Designer version 4.1 (Micrografx Inc., Richardson, TX, USA), and SPSS version 12 (IBM Inc., Armonk, NY, USA).

Table 2.—Impacts of spatial, temporal and landscape variables on herb-dwelling spiders: Durbin-Watson statistics (D-W stat), R^2, and significance of the whole model (Sign. model). The figures in the predictor lines indicate the standardized coefficients (beta) and the significance levels (* ≤ 0.05, ** ≤ 0.01), respectively. N_{spec} = total number of species, dohs = number of species preferring dry open habitats, Acu_cero = *Aculepeira ceropegia*, Aga_reed = *Agalanatea reedii*, Ara_quad = *Araneus quadratus*, Arg_brue = *Argiope bruennichi*, Mic_pusi = *Microlinyphia pusilla*, Liny_tot = total number of linyphiid individuals, Area = area of set-aside, TSet-aside = time since set-aside, TLastMan = time since last management activities, Vheight = mean (of four sampling dates) of the vegetation height, VCover = mean of vegetation cover, D_field = distance to the nearest arable field, D_wat = distance to the nearest waters, D_reed = distance to the nearest reed, D_hedgerow = distance to the nearest hedgerow, D_wood = distance to the nearest wood. The variables that showed significance slightly above the level of p = 0.05 are given in brackets.

Parameters	Nspec	dohs	Acu_cero	Aga_reed	Ara_quad	Arg_brue	Mic_pusi	Liny_tot
D-W stat	2.12	2.24	2.24	2.30	1.27	2.12	1.83	1.93
R^2	0.53	0.55	0.67	0.66	0.70	0.67	0.57	0.64
Sign. model	0.05	0.04	0.002	0.003	0.001	0.003	0.02	0.006
Area [ha]	0.78, *	0.79, *.	0.83, **	0.59, *	0.16, n.s.	0.91, **	0.17, n.s.	0.28, n.s.
TSet-aside [days]	0.02, n.s.	0.15, n.s.	0.40, n.s.	0.40, n.s.	−0.005, n.s.	−0.15, n.s.	−0.17, n.s.	−0.20, n.s.
TLastMan [days]	0.26, n.s.	0.27, n.s.	−0.20, n.s.	0.39, n.s.	−0.31, n.s.	−0.10, n.s.	−0.08, n.s.	0.02, n.s.
Vheight [cm]	0.53, **	0.13, n.s.	0,47,**	0.07, n.s.	0.68, ***	0.33, *	−0.25, n.s.	−0.20, n.s.
VCover [%]	0.08, n.s.	0.11, n.s.	−0,46,*	−0.10, n.s.	0.36, *	0.54, **	0.36, (*)	0.42, *
D_field [m]	0.02, n.s.	0.12, n.s.	0.06, n.s.	−0.02, n.s.	0.32, (*)	0.39, *	0.22, n.s.	0.30, n.s.
D_waters [m]	0.26, n.s.	0.38, *	0.21, n.s.	0.04, n.s.	0.20, n.s.	0.14, n.s.	0.05, n.s.	0.09, n.s.
D_reed [m]	−0.15, n.s.	−0.23, n.s.	−0,37, *	−0.20, n.s.	0.23, n.s.	0.24, n.s.	−0.52, *	−0.52, **
D_hedgerow [m]	0.21, n.s.	0.16, n.s.	−0.05, n.s.	0.16, n.s.	0.19, n.s.	0.16, n.s.	−0.10, n.s.	−0.11, n.s.
D_wood [m]	−0.56, n.s.	−0.47, n.s.	−0,56, *	−0.40, n.s.	0.27, n.s.	−0.18, n.s.	−0.46, n.s.	−0.53, *

RESULTS

Composition of spider assemblages.—In all, 23 species and 7,323 individuals were identified, including 3,090 adults of 23 species and 4,233 juveniles of 18 species. The adult and juvenile species' abundance and their preferred habitat types are listed in Table 1; the species and ecological group tested, along with the number of individuals of adult and juvenile spiders combined can be found in Table A2.

In accordance with the sampling method used, most of the species belonged to the families Araneidae, Linyphiidae and Theridiidae. The plurality of species (39.1%) were characteristic for fallowland, followed by those species preferring dry forests (including dry forest edges) and arable fields at 17.4% and 13.0%, respectively. The fallowland species contributed 66.3% to the total number of individuals, and three species that preferred arable fields accounted for 21.5%. The ratio of adult to juvenile individuals for the species that prefer arable fields was 1:39.8, while the corresponding ratio for the species that prefer dry open habitats was 1:0.8 (see Table 1).

Variables influencing overall species richness.—The results of the multiple linear regression are presented in Table 2. The most influential factor that significantly increased the total number of species at the set-aside plots was vegetation height (Table 2). In contrast, vegetation cover revealed no significant effect on species richness. Another factor that increased the number of species significantly was the area of the plots. Neither the time since establishment, the time since the last management activities at the set-asides nor the distances to any of the surrounding habitat types showed a significant effect on the number of species.

Variables influencing the number of individuals of particular species and of the ecological group.—The number of species that prefer dry open habitats increased significantly as the distance to waters increased. Vegetation height and cover and temporal factors revealed no significant effects. Area was the most influential factor that significantly increased the number of species of the "dry open habitats" ecological group.

In addition to area, vegetation height was the most influential factor that increased the number of individuals of the araneid species *Aculepeira ceropegia* (Walckenaer 1802), with a high significance on the local scale. However, with increasing vegetation cover, the number of individuals of *A. ceropegia* significantly declined. On the landscape scale, a significant negative relationship was found between the number of individuals of this species and the distance to woods or waters (see Table 2).

The only predictor that signifieantly inereased the number of individuals of *Agalenatea redii* (Scopoli 1763) was the area of the set-aside plots.

Vegetation structure (height and cover) was the most significant predictor increasing the abundance of *Araneus quadratus* Clerck 1757. Neither area nor temporal factors had a significant influence on the abundance of this species. On the landscape scale, the abundance of *A. quadratus* rose with greater distance to arable fields (significance slightly above 0.05).

A significant increase in the abundance of individuals with greater distance to an arable field was also found for *Argiope bruennichi* (Scopoli 1772). On the local scale, the number of individuals of this species increased highly significantly as vegetation cover became more dense, and increased significantly with vegetation height. However, the most influential factor that positively influenced the abundance of this species was the area of the plot.

The number of individuals of the linyphiid family in general and for *Microlinyphia pusilla* (Sundevall 1830) in particular significantly benefited from dense vegetation cover. None of the remaining factors on the local scale had a significant influence on the number of individuals. On the landscape scale there was a strongly significant negative relationship between the distance to reeds and the number of individuals and a significant negative relationship between the distance to woods and the abundance of the linyphiid species as a whole. For *M. pusilla* the same negative relationship was merely significant for the distance to reeds (see Table 2).

DISCUSSION

The total number of species and the number of species that prefer dry open habitats significantly increased with the increasing area of the set-asides. This observation can also be extended to the abundances of most of the araneid species tested, but there was no such significant relationship observed with regard to the linyphiid species in general and *M. pusilla* in particular. These findings imply that larger set-asides may provide more structural diversity for araneid species to meet their different requirements for web building, overwintering and dispersal (Rypstra et al. 1999; Bell et al. 2001). The number of individuals of the much smaller linyphiid species, which build small webs near the ground, appears to depend primarily on the availability of dense vegetation cover and shows no significant relationship with the area of the plots. Nevertheless, this finding supports the more general conclusion of van Buskirk & Willi (2004) whose meta-analysis of studies on the beneficial impact of set-aside areas stressed that spider density increases markedly as the area of the set-aside increases (from 0.002 to 50 ha).

Our findings show that there is no significant relationship between time since the set-aside was established or time since the last management and total number of species or number of individuals of particular species and the preferred habitat type. The corresponding findings of comparable studies are rather inconsistent. Bell et al. (1998) investigated the ground-dwelling spider communities of regenerated disused quarries and found no relationship between the number of species and individuals and the age of the sites. Furthermore, these authors found that the number of species and individuals did not differ between highly managed and unmanaged sites. In contrast, Frank et al. (2009) stressed that the density, biomass and speeies richness of spiders increased as the age of wildflower sites increased from one to four years, and Gibson et al. (1992a) found a net increase of species richness over a sampling time of six years in grazed grasslands. Van Buskirk & Willi (2004) found that the benefit represented by the density of spiders in set-aside areas varied with the number of years since the land was removed from conventional production and showed a strong increase in the first six years since establishment. Tscharntke et al. (2011) demonstrated that the species richness of different animal groups was the highest in two year-old set-aside fields in a sequence from one- to three-year-old set-asides; no further significant increase in the species richness was found in the

older set-asides. If we view the conclusions of Tscharntke et al. (2011) as generally accepted, we recognize that significant increases in the species richness and in the number of individuals were unlikely to occur in our study because all of the plots examined had attained or exceeded an age of two years (see Appendix, Table A1).

Our results show that, in addition to area, vegetation structure (height and cover) is the most influential predictor in relation to the benefits, as measured by the total number of species, by linyphiid individuals and by most of the araneids. In conjunction with the findings above, after two years of succession from pioneer to at least ruderal vegetation, there are no further significant changes in the abundance and number of species in the spider communities of the herb layer. Two of the araneid species benefited from both a high and dense vegetation cover, whereas the number of individuals of one species (*Aculepeira ceropegia*) increased with a high vegetation cover but decreased with a dense vegetation cover. However, the number of linyphiid individuals only significantly profited from dense vegetation cover. These results are generally consistent with the findings by Frank et al. (2009) that the number of individuals of spider assemblages was best explained by the vegetation cover. Several authors emphasize the significance of a richly structured vegetation cover for herb-dwelling spiders (Uetz 1991; Robinson 1981). However, a rich structure may occur within both high (Rypstra et al. 1999; Bell et al. 2001) and low vegetation covers (Gibson et al. 1992b; Bell et al. 2001). Moreover, our results demonstrate that the structure is not beneficial for herb dwelling-spiders as a whole but is used by different spider species and families in different ways.

The number of individuals of two of the araneid species caught was positively related to the distance to the adjacent crop habitats. This finding may indicate that the set-asides are not originally colonized from arable fields. This assumption is supported by Hatley et al. (1996), Samu et al. (1999), Schmidt et al. (2005), and Thorbek & Topping (2005) who found that a higher proportion of non-crop habitats in the surrounding landscape was associated with increases in the number of spiders in cereal fields. In summary, our results support those of other empirical studies in agroecosystems (Duelli & Obrist 2003; van Burskirk & Willi 2004) and the theoretical considerations in Hanski (1998) that set-asides may benefit from the proximity of appropriate colonization sources but may also act as a source of colonists for other set-asides and secondarily for the surrounding landscape. Therefore, our results stress the complex effect of the landscape matrix bordering set-asides on the herb-dwelling spider species.

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APPENDIX

Table A1.—Spatial and temporal variables at the plots investigated. Veg Height = vegetation height (mean of four sampling dates), Veg Cover = vegetation cover (mean of four sampling dates), TSet-aside = time since set-aside of the plot, TLastMan = time since the last management activity at the plot, ManFrequ = management frequency from 1999 to 2001. Distances to the nearest adjacent habitat types; 1111 denotes that the habitats were more than 1000 m apart from the set-asides. The plots are named after the nearby villages in northern Uckermark, Fredersdorf (Fr), Gollmitz (Go), Güstow (Gu), Naugarten (Na), Stendell (St), and in southern Uckermark, Passow (Pa), Polßen (Po), Welsickendorf (We), and Zichow (Zi), and the Müncheberg area (Mu). The numbers immediately following "Gu 23" refer to different lots.

Plots	Area [ha]	Veg Height [cm]	Veg Cover [%]	TSet-aside [yrs]	TLast Man [yrs]	Distance to nearest adjacent arable field [m]	Distance to nearest hedgerow [m]	Distance to the nearest waters [m]	Distance to the nearest reed [m]	Distance to the nearest wood [m]
Fr2	2.37	125.0 ± 23.8	98.8 ± 2.5	2.98	2.98	0	0	30	80	1111
Go2	1.57	92.5 ± 27.5	86.3 ± 18.0	1.97	1.85	0	660	255	205	1111
Gu1	0.56	110.0 ± 26.5	96.7 ± 5.8	2.97	1.24	0	30	1111	95	215
Gu2	0.41	120.0 ± 34.6	86.7 ± 5.8	2.97	2.97	0	0	990	17	290
Gu4	0.16	70.0 ± 26.5	90.0 ± 10.0	2.97	0.24	0	0	355	730	0
Gu7	0.11	76.7 ± 25.2	100.0 ± 0	2.97	0.21	0	310	80	120	0
Gu8	0.37	83.3 ± 5.8	93.3 ± 11.5	2.98	1.24	0	0	20	400	340
Gu11	0.92	120.0 ± 16.3	100.0 ± 11.5	2.97	2.97	0	135	150	60	1111
Gu23.1	0.30	130.0 ± 26.5	86.7 ± 11.5	1.98	1.98	110	260	180	110	640
Gu23.2	0.21	82.5 ± 25.0	62.5 ± 18.9	1.97	1.97	100	110	210	510	425
Gu23.3	0.14	46.7 ± 5.8	87.7 ± 4.0	1.97	1.97	150	195	165	495	305
Gu23.4	0.15	100.0 ± 34.6	77.5 ± 28.7	1.97	1.97	150	180	165	360	385
Gu23.5	0.19	66.7 ± 5.8	78.3 ± 10.4	1.98	1.98	175	270	255	315	215
Gu23.6	0.18	53.3 ± 5.8	80.0 ± 0	1.98	1.98	160	175	310	305	245
Gu23.7	0.28	113.3 ± 5.8	95.0 ± 5.0	1.98	1.98	75	170	180	160	490
MuB1	4.58	62.5 ± 20.6	87.5 ± 12.6	9.96	2.17	0	0	0	0	1111
MuB8	4.62	115.0 ± 17.3	100.0 ± 0	9.96	9.96	285	145	60	50	1111
Mu1	4.90	85.0 ± 20.8	57.5 ± 25.0	2.96	2.96	0	185	235	150	1111
Mu3	5.26	65.0 ± 12.9	67.5 ± 22.2	2.96	0.11	30	30	25	0	1111
Mu4	5.60	82.5 ± 28.7	100.0 ± 0	2.96	0.19	0	0	0	0	1111
Mu5	5.54	80.0 ± 36.5	100.0 ± 0	4.96	0.17	0	0	0	20	1111
Mu9	5.22	75.0 ± 34.2	97.5 ± 5.0	3.96	0.80	0	5	120	115	1111
Mu22	4.61	57.5 ± 5.0	75.0 ± 12.9	1.96	1.84	0	260	345	70	1111
Na1	1.10	57.5 ± 9.6	65.0 ± 28.9	1.97	1.91	0	380	655	370	350
Pa1	3.69	100.0 ± 27.1	80.0 ± 16.3	1.98	1.86	0	250	410	1111	1111
Po1	3.24	90.0 ± 24.5	90.0 ± 8.2	2.98	2.98	0	0	0	75	1111
Po3	3.35	100.0 ± 14.1	92.5 ± 5.0	9.98	9.98	0	55	310	120	1111
Po5	3.23	90.0 ± 29.4	70.0 ± 8.2	2.98	2.86	0	30	10	280	1111
St1	3.81	70.0 ± 30.0	63.3 ± 37.9	2.98	2.86	130	500	715	1111	755
St4	3.90	103.3 ± 47.3	76.7 ± 25.2	9.98	1.13	90	0	10	1111	0
We4	2.61	57.5 ± 15.0	92.5 ± 15.0	9.98	1.11	0	215	550	1111	1111
Zi3	2.05	50.0 ± 0	79.5 ± 13.7	1.98	0.08	0	20	50	245	480

Table A2.—Numbers of individuals (sum of five sampling points at each plot over 4 months) of araneid and linyphiid species (adults and juveniles combined) used in analysis and the number of species preferring dry open habitats (dohs) at the investigated plots. See Table A1 and Table 2 for the abbreviations of the plots and species names, respectively.

Plots	Acu_cero	Aga_reed	Ara_quad	Arg_brue	Mic_pusi	Liny_tot	dohs
Fr2	6	3	29	4	108	114	6
Go2			15	3	20	20	5
Gu1	7		17		127	127	4
Gu2	11	1	6	3	62	92	9
Gu4	8		3	2	20	25	5
Gu7			2		213	218	3
Gu8	8				125	130	4
Gu11			1		53	58	4
Gu23.1	7		11	3	78	84	5
Gu23.2	2		4		23	23	4
Gu23.3					121	121	4
Gu23.4	5		6	1	51	51	4
Gu23.5	2		3		253	261	4
Gu23.6			1	4	464	464	4
Gu23.7	1		38	6	83	83	3
MuB1	52	23	2		47	47	6
MuB8	16	19	37	71	40	118	9
Mu1	41	2	1	2	40	42	6
Mu3	15				23	25	5
Mu4	6	1	1	9	100	100	6
Mu5	9		10	7	101	101	4
Mu9	4		5	28	162	163	7
Mu22	35	16	2	6	168	171	6
Na1	6				53	55	4
Pa1	2		53	5	9	12	3
Po1			3		52	52	3
Po3	3	11			46	46	8
Po5	1				43	43	2
St1	5	4	2	2	16	16	8
St4	26	7	2	7	72	72	6
We4			7	8	20	20	4
Zi3					46	46	3

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Habitat use in an assemblage of Central American wandering spiders

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Abstract. The sympatric occurrence of species is thought to be based mainly on the differences in their use of habitat and of limiting resources. Segregating parameters may be of spatial or temporal character and may include behavioral differences. We hypothesized that species of large hunting spider living sympatrically in a Costa Rican lowland rain forest should differ in their habitat and/or hunting microhabitat preferences, in daily activity pattern, and, as an adaptation to the preferred hunting microhabitat, in their specific ability to adhere to smooth surfaces. We found an assemblage of eight large species of the families Ctenidae and Trechaleidae, consisting of three subguilds: 1) two semi-aquatic species with low adhesion ability, 2) three forest-floor dwelling species with good adhesion ability, and 3) three vegetation dwelling species showing very good adhesion ability. The species were partially segregated by habitat type, with two of the vegetation dwelling species preferring the treeless area of a temporary swamp. We found no species-specific differences in daily activity patterns. The similarity in community structure between this Costa Rican and a central Amazonian assemblage suggests the existence of similar structuring mechanisms in wandering spider assemblages in climatically similar biomes.

Keywords: Coexistence, habitat preference, natural prey, niche, sympatry, Ctenidae, Trechaleidae

Niche theory suggests that coexistence of similar species is based on differences that allow a species-specific use of limited resources, thus avoiding or diminishing competition within animal communities (Putman 1994). Such differences may also be based on spatial (or temporal) aspects: spiders not only can use different ways to capture prey, but also can hunt in different places (or at different times) for the same type of prey in a similar way. As abundant and mostly unspecialized predators, spiders are important elements of many terrestrial animal communities (Wise 1993; Pfeiffer 1996; Hurtado Guerrero et al. 2003; Sørensen 2003). Wandering spiders, specifically spiders not using webs for prey capture and therefore having low site fidelity, may have high population densities and a considerable impact on arthropod communities (Wise 1993; Sørensen 2003). Although many tropical habitats harbor many of these species, they are rarely studied.

Local assemblages may be composed of several large species that on first glance appear to be quite similar to each other in morphology and hunting behavior. However, similar sympatric species frequently differ in microhabitat and diet preferences and, less frequently, also in temporal activity patterns (e.g., Mühlenberg 1993; Moring & Stewart 1994; Menin et al. 2005). Nevertheless, niche width may overlap to some extent (Putman 1994), especially when eonsidering largely unspecialized predators such as spiders. To assess the community eeology of large araneomorph wandering spiders, we studied patterns of habitat segregation within a Costa Rican lowland forest assemblage composed of similarly sized species of the families Ctenidae and Trechaleidae.

The pantropically distributed Ctenidae family (superfamily Ctenoidea: Silva Davila 2003) contains some of the largest araneomorph wandering spider species, with body lengths of more than 4 cm. In tropical lowland habitats, these spiders are often found in assemblages consisting of several large species. Frequently, these large spiders occur sympatrically with the New World endemic family Trechaleidae (superfamily Lycosoidea: Silva Davila 2003) that contains species of similar size to the Ctenidae (Carico 1993). Although some studies have focused on various aspects of selected tropical wandering spider taxa (Van Berkum 1982; Barth et al. 1988; Carico et al. 1985; Schmitt et al. 1990; Carico 1993; Höfer et al. 1994; Gasnier & Höfer 2001; Steyn et al. 2002; Dias & Brescovit 2004; Torres-Sánchez & Gasnier 2010), no studies on community patterns within assemblages of similarly sized sympatric species belonging to different families are yet available. Consequently, our goal was to make an ecological analysis of an assemblage of large wandering spider species. We quantified habitat parameters in order to assess patterns of habitat use. We hypothesized that species should differ from each other in habitat and/or microhabitat choice, and that species with preferences for different microhabitats should also differ in their ability to adhere to smooth surfaces. Plant dwellers should have better adhesive abilities than ground dwellers because they often move on vertical surfaces and on the undersides of smooth leaves. Additionally, we expected different diurnal activity patterns of the species.

METHODS

Study site.—We conducted field work at the Reserva Biológica Tirimbina (RBT; 10°24′N, 84°07′W, 180–220 m asl), Heredia Province, Costa Rica, comprising an area of 345 ha adjacent to the Sarapiquí River. Mean annual temperature is 25.3°C and mean annual precipitation is 3777 mm. Near the Sarapiquí River lies a temporary swamp that is partly covered by forest; however, its main area lacks trees and is densely covered with tall (up to 3.5 m) grass, vines, *Heliconia* spp. (Heliconiaceae) and Maranthaceae up to 6 m tall. RBT includes areas belonging to two life zones: very humid, tropical, pre-montane forest and transitional very humid tropical forest (Holdridge 1967). Eighty-five per cent of

the reserve's forest is classified as "primary forest." RBT also encompasses areas of secondary forest of various age classes and an abandoned cacao plantation with relatively short (ca. 6–10 m) cacao trees, with some taller shade trees and very little undergrowth, surrounded by forest (Reserva Biológica Tirimbina 2009).

Sampling.—To obtain data on habitats and hunting microhabitats, we searched for active subadult and adult spiders over the course of 50 nights, from 1830 h to 0550 h (22 April to 10 July 2008). All spiders encountered at the entrance or outside of their day shelters were considered to be active. Only large species with a body length ≥ 17 mm were surveyed. We identified species using the literature available (Pickard-Cambridge 1897, 1897–1905; Carieo 1993; Höfer & Breseovit 2000; Barth 2001; Simó & Brescovit 2001). Voucher specimens were preserved in ethanol (70%) and deposited in the Museo de Zoología, Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica.

Habitat, hunting microhabitat and activity: We conducted most of the fieldwork in the western part of RBT along trails, two creeks, two canopy bridges (height = 0–24 m) and within a temporary swamp. Although the canopy bridges allowed us to detect spiders up to 30 meters above ground, most individuals on higher tree trunks and branches could not be reached. We obtained the majority of the data from the forest floor and the understory. Spiders were marked individually on the carapace with small, numbered, plastic tags used in beekeeping in order to avoid repeated sampling. We attached the tags to the carapace by non-water-soluble Tippex ™ (modified after Azevedo 1999). Date and time of each encounter were recorded. We distinguished the following habitat types: forest, gap, forest margin, temporary swamp (treeless part), and cacao plantation.

In order to characterize hunting microhabitat preferences, we recorded the following parameters for each individual: type of hunting microhabitat (with the classes: st = stone, so = soil, wa = water, lo = log, vr = vines and thin hanging roots, br = branch, le = leaf, pest = petiole of a leaf of a palm/stem of a tall grass, tru = tree trunk), height above ground (HG, in m), distance to the nearest water body (DW, in m), temperature close to the spider (T, in °C, precision 0.1°C), and angle of inclination of the substrate it sits on (α, in degrees °C). We also recorded degree of cover (DC) by epiphylls, grass or leaf litter around an individual, as it can provide shelter or obstruct the locomotion of relatively small animals such as spiders, thus altering the preferred surface structure among species. We estimated the degree of cover around an individual spider using a transparent plastic sheet of 58 × 40 cm with a grid of 2 × 2 cm squares. Height of cover around the spider (HC, in cm) was scored as the mean of six measurements: four in a distance of 10 and 20 cm in front of and behind the spider, respectively, and two 10 cm to the right and the left of the spider.

Prey: Whenever one of our focus species encountered a prey item, we tried to identify it at least to ordinal level. The aim was to verify an overlap of diet among the spider species studied. Body mass of prey was recorded using a digital portable balance (Acculab, Sartorius Group, Pocket Pro–PP 62, accuracy 0.01 g). As prey data were scarce during fieldwork in 2008 (n = 37), we added data collected between July 2010 and March 2012.

Table 1.—Co-occurring species of large wandering spiders within RBT. The numbers of individuals for each gender include both subadults and adults, except that for *P. boliviensis,* two females of a stage prior to the subadult stage were included.

Species	Females	Males	Sum
Ancylometes bogotensis (Keyserling 1877)	13	9	22
Phoneutria boliviensis (F.O.P.-Cambridge 1897)	3	3	6
Cupiennius coccineus F.O.P.-Cambridge 1901	43	23	66
Cupiennius getazi Simon 1891	12	10	22
Ctenus sinuatipes F.O.P.-Cambridge 1897	17	6	23
Ctenus curvipes (Keyserling 1881)*	13	7	20
Ctenus sp. 3	19	14	33
Trechalea tirimbina Silva & Lapinski 2012	14	13	27
Total	134	85	**219**

* New species record for Costa Rica.

Adhesion ability and body mass: The animals' ability to adhere to smooth surfaces such as leaves was experimentally quantified in order to assess whether it corresponded to the surface of the preferred hunting microhabitat. We used plexiglass as a standardized smooth surface in order to test the individuals under constant and reproducible conditions. Each spider was placed on a plexiglass square (20 cm × 20 cm). The angle of inclination (β) of this square was then slowly increased in steps of 45°, so that the position of the spider changed from being on the upper side (0 and 45°), to hanging on a vertical surface (90°), to finally clinging to the underside of the plexiglass (135 and 180°). The largest angle at which the spider was still able to stay attached to the plexiglass was reeorded. Body mass of each spider (m) was assessed using a portable digital balance (see above).

Statistical analyses.—In order to show the overall assemblage pattern and the position of microhabitat variables and adhesion ability relative to each other and to the centroids of the speeies, we eonducted an unrotated Principal Component Analysis (PCA) using Statistica (Version 6.0). The data were $\log_{10}(x+1)$–transformed and standardized. For each microhabitat type, we used presence/absence data of each spider species, the mean values of HG, DW, T, α, DC and HC measured near the spiders and of β of the spiders in the respective microhabitat type (see above). We then used SigmaStat (Version 3.5) to test for interspecific significant differences. We tested for interspeeific differences in use of microhabitat classes (nominal variables) with a Chi-square test. For continuous variables, analysis of variance (ANOVA) was applied to normally distributed and Kruskal-Wallis ANOVA to non-normally distributed data. Dunn's method was used as a post-hoc test, to compare data that were not normally distributed and were of unequal size.

RESULTS

Species composition.—We found 219 large spiders belonging to seven species of the family Ctenidae and one species of the family Trechaleidae (Table 1). Median body mass of female

Table 2.—Prey of the spider species studied. Species abbreviations: Ab = *Ancylometes bogotensis*, Cc = *Cupiennius coccineus*, Cg = *Cupiennius getazi*, Cts = *Ctenus sinuatipes*, Ctc = *Ctenus curvipes*, Ct 3 = *Ctenus* sp. 3, Pb = *Phoneutria boliviensis*, Tt = *Trechalea tirimbina*.

	Predators							
Prey	*Ab*	*Cc*	*Cg*	*Ctc*	*Cts*	*Ct 3*	*Pb*	*Tt*
Arachnida								
Amblypygi						1		
Araeneae	1	3	2		1	1	3	4
Opiliones		1		1				
Scorpiones		1						
Insecta								
Blattodea				2				
Dermaptera		1						
Heteroptera			1					
Homoptera		1			1			
Hymenoptera							1	
Lepidoptera		1						
Odonata			1					
Orthoptera		5	2	1	2		2	4
Phasmida		1						
Scolopendromorpha				1	1	1		
Vertebrata								
Anura		1		1				
Cyprinodontiformes	1							
Squamata				1				
Totals	2	15	6	7	5	3	6	8

spiders ranged between 0.68 g [*Ctenus curvipes* (Keyserling 1881)] and 2.59 g [*Ancylometes bogotensis* (Keyserling 1877)]; males ranged between 0.73 g (*Ct. curvipes*) and 2.31 g [*Phoneutria boliviensis* (F.O.P.-Cambridge 1897)]. No other araneomorph wandering spider species of similar size were observed at the study site.

Prey.—During both field trips, we observed spiders with prey that could be identified at least to ordinal level in 52 cases. Seventeen taxa (suborder or order) were observed as prey of the spiders, including three vertebrate orders. Other spiders (Araneae) and orthopterans (mainly crickets and katydids) were consumed by most of the spider species studied (Table 2). During our first field trip, the body mass of the prey items (n = 37) did not differ significantly among the spider species (Fig. 1; ANOVA: $F_{7,29}$ = 1.2, P = 0.32).

Overall assemblage structure.—The PCA of the presence/absence data of the spider species, hunting microhabitat types, and adhesion ability shows a structured assemblage (Fig. 2). The eigenvalues were 4.06 (1st axis) and 1.68 (2nd axis). These two principal components explained 81.9% of the total variance in the data set.

The PCA suggested that the presence of *A. bogotensis* and *Trechalea tirimbina* Silva & Lapinski 2012 was negatively correlated with distance to a water body (DW), height above ground (HG), degree of cover (DC), and height of cover (HC). These species appear to be associated with the microhabitat types of water, stone, log, and vines and thin hanging roots. The presence of the three *Ctenus* species appeared to be positively correlated with the temperature of the hunting

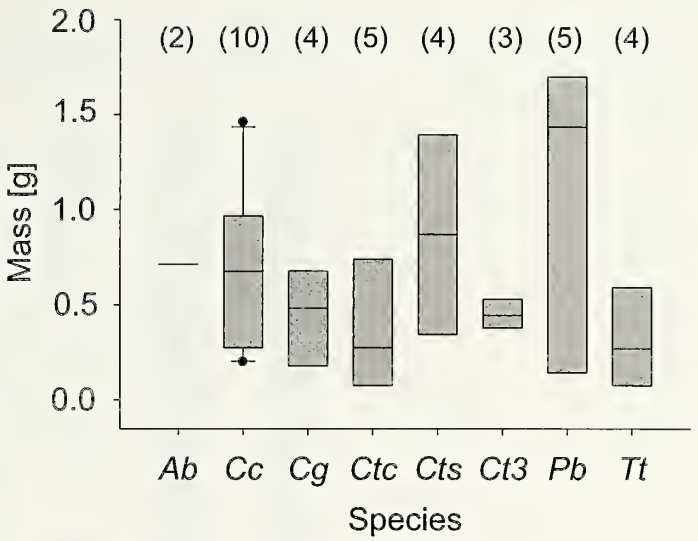

Figure 1.—Box plot of mass of prey items captured by the spider species. For species abbreviations see legend to Table 2. Numbers in brackets indicate sample size.

microhabitat (T), DC and HC, and DW (less so for *Ctenus* sp. 3), but negatively correlated with α. Those species appear to be associated with the following microhabitat types: soil, tree trunk, and branch. PCA suggested a positive correlation of the presence of *Cupiennius coccineus* F.O.P.-Cambridge 1901 with DW, HG, and an association with the microhabitat types of tree trunk, branch and leaf. Presence of *Cupiennius getazi* Simon 1891 and *P. boliviensis* appeared to be positively correlated with α and HG, and negatively with T, DC and HC. The analysis suggested an association of those species with the

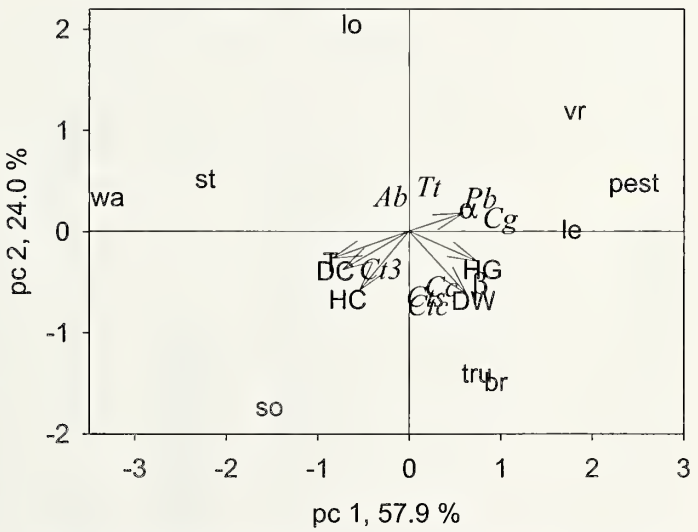

Figure 2.—Principal component analysis (PCA) biplot showing the overall assemblage structure at RBT. Abbreviations: st = stone, so = soil, wa = water, lo = log, vr = vines and thin hanging roots, br = branch, le = leaf, pest = petiole of a leaf of a palm/ stem of a tall grass, tru = tree trunk, HG = height above ground, DW = distance to the nearest water body, T = temperature close to the spider individual, α = inclination of the substrate a spider perches on, DC = degree of cover, HC = height of cover, β = angle of the plexiglass square, i.e. adhesion ability. For species abbreviations see legend in Table 2.

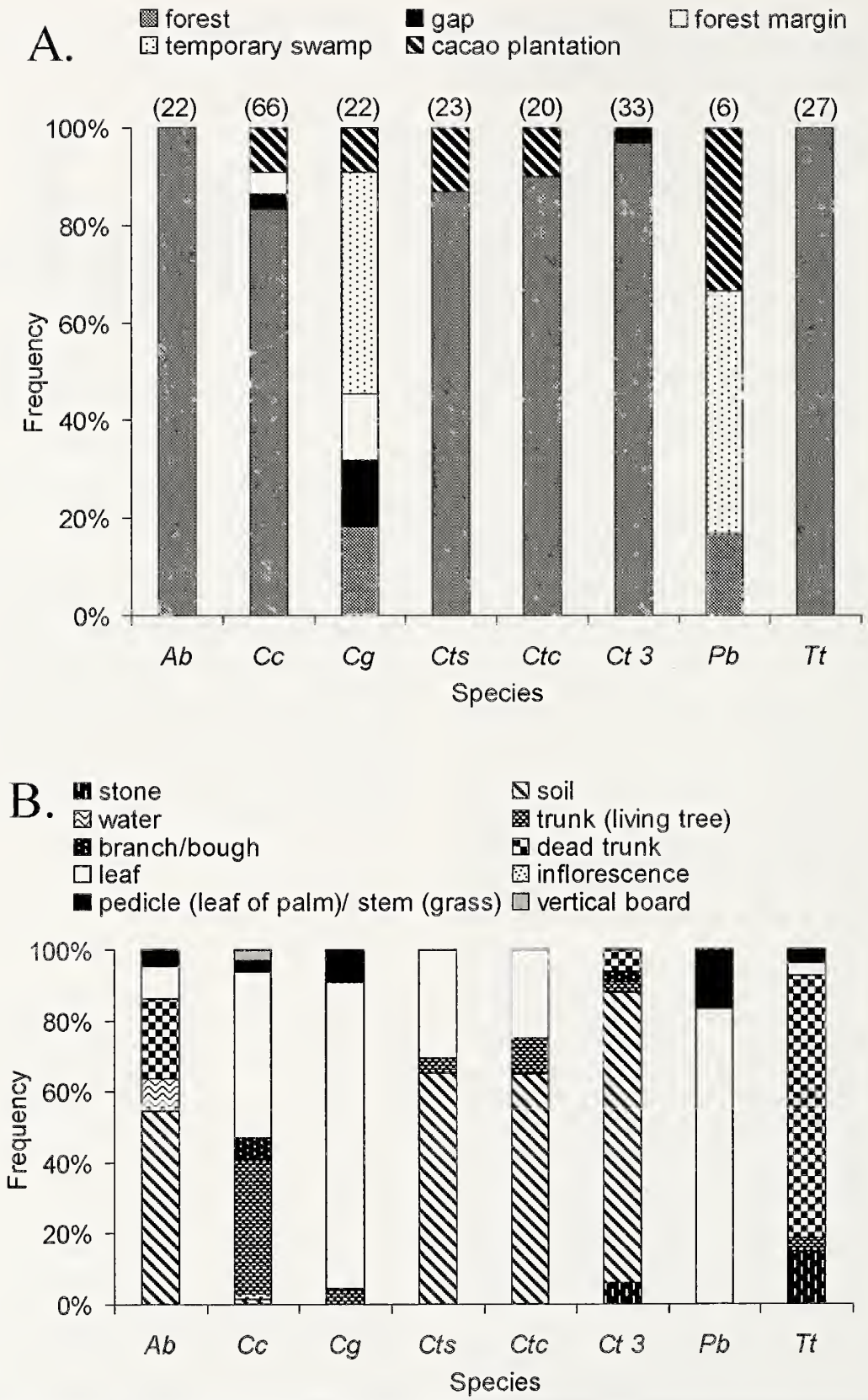

Figure 3.—Relative frequency of occurrence of the spider species at (A) habitat types and at (B) hunting microhabitats. Numbers in brackets indicate sample size. For species abbreviations, see legend in Table 2.

microhabitat types of vines and thin hanging roots, petiole of a leaf of a palm/stem of a tall grass, and leaf.

Distribution, habitats and activity.—The species occurred in five different habitat types (Fig. 3A). Eighty-two per cent of all individuals were found within the forest. *Ancylometes bogotensis* and *T. tirimbina* were present only along creeks within the forest. *Cupiennius coccineus* and the three species of *Ctenus* were widely distributed over the entire forest and appeared less frequently in the eacao plantation. *Cupiennius getazi* and *Phoneutria boliviensis* occurred mostly in treeless areas, especially on the vegetation within and around the swamp. *Cupiennius getazi* also inhabited all other habitat types, but in very low numbers. *Cupiennius coccineus*, the most abundant species overall (Table 1), was never found in open areas. Individuals of all species were encountered outside their day shelters throughout the night, and we found no specific preferences in activity time (Kruskal – Wallis ANOVA: H_7 = 10.0, P = 0.19).

Hunting microhabitat.—The spider species differed significantly in the microhabitat type on which they were found hunting (χ^2 test: χ^2_{56} = 322.3, $P < 0.001$; Fig. 3B). Many spiders frequented leaves, especially both species of *Cupiennius* and *P. boliviensis*, although *C. coccineus* mainly used trees and palms, while *C. getazi* and *P. boliviensis* were found on very high grass and on *Heliconia* sp. In eontrast to these three species, *A. bogotensis* and the three *Ctenus* species were found mostly on the forest floor, and only some *Ctenus sinuatipes* F.O.P.-Cambridge 1897 and *Ct. curvipes* also hunted on leaves of the lower forest vegetation. *Trechalea tirimbina* mainly used logs and stones. The distance of the respective hunting microhabitats from water (DW) differed signifieantly among the species, with *A. bogotensis* and *T. tirimbina* almost always occurring in the immediate vicinity of creeks and small rivers. *Ctenus* sp. 3 was also found very often near bodies of water (Kruskal-Wallis ANOVA: H_7 = 103.2, $P < 0.001$; Dunn's post-hoc test: $P < 0.05$: Fig. 4A). Heights above ground of the hunting microhabitats differed significantly between the spider species (Kruskal-Wallis ANOVA: H_7 = 153.7, $P < 0.001$; Dunn's post-hoe test: $P < 0.05$; Fig. 4B). The two *Cupiennius* species and *P. boliviensis* occurred mainly higher above the ground than the other ctenids and *T. tirimbina*.

Surfaee cover near the spiders on plants, logs, and rocks in the creeks consisted mainly of small epiphytes. On soil, it was predominantly composed of litter and smaller plants. Low to very low DC and HC values were found near the two *Cupiennius* species, *P. boliviensis* (in all three species: median DC = 0%, median HC = 0.0 cm), and *T. tirimbina* (median D_C = 15%, median H_C = 0.1 cm). High DC and HC values were found for hunting microhabitats of *A. bogotensis* and *Ctenus* sp. 3 (median DC: 40 and 39%, median HC: 1.6 and 2.2 cm, respectively). Very high DC and HC values mainly occurred in microhabitats of *Ctenus sinuatipes* and *Ct. curvipes* (median DC: 80 and 79%, median HC: 2.2 and 2.8 cm, respectively). Both parameters in the latter four species were very variable (DC = 0–100%, HC = 0.0–12.0 cm). *Ctenus sinuatipes* and *Ct. curvipes* were found in microhabitats with significantly higher DC values than the two *Cupiennius* species and *P. boliviensis*. *Ctenus curvipes* and *Ctenus* sp. 3 preferred significantly higher HC values than the two *Cupiennius* species and *P. boliviensis*. *Trechalea tirimbina* hunted in microhabitats with significantly lower HC values than *Ctenus* sp. 3. (Kruskal-Wallis ANOVA: H_7 = 55.7 for DC and H_7 = 77.3 for HC, P = 0.001; Dunn's post-hoc test: $P < 0.05$; Fig. 4C–D). Temperature near individual spiders did not differ significantly among species (Kruskal-Wallis ANOVA: H_7 = 10.6, P = 0.16; Fig. 4E).

Adhesion ability.—The PCA suggested a positive correlation of β with DW and HG and proved to be characteristic for species having high HG- and/or DW- values (Fig. 2). *Ancylometes bogotensis* and *T. tirimbina* had significantly lower adhesion ability than the other species. Although these species barely reached values of 45° in the adhesion experiments, some of the others were able to cling to the plexiglass even when turned upside down (β =180°) (Kruskal-Wallis ANOVA: H_7 = 131.7, $P < 0.001$; Dunn's post-hoc test: $P < 0.05$; Fig. 4F).

DISCUSSION

We have presented a comparative study of the ecology of an assemblage of large tropical araneomorph wandering spiders comprising two families, Ctenidae and Trechaleidae. Subadults and adults of the different species preyed on a similarly sized group of animals. Even the smallest and lightest species, *Ctenus curvipes,* was found with prey of considerable body mass that overlapped widely with the prey of all other species. Although data on prey are limited, we conclude that the large wandering spiders at the study site form an ecological guild, defined by the use of the same resources (Root 1967). However, our data also show that the eight species are almost entirely segregated by different habitat use, and within overlapping habitat sections they differed in microhabitat choice.

Habitat segregation was most noticeable for *C. getazi* and *P. boliviensis*, with a preference for treeless habitats. The presence of *C. getazi* and the absence of *C. coccineus* from such habitats were also reported by Schuster et al. (1994). The close proximity to water of *A. bogotensis* and *T. tirimbina* corroborates the reported strong association of these two genera with water (Carico 1993; Höfer & Brescovit 2000), separating them from the other six species.

Contrary to our expectation, all species were found throughout the night. This confirms the lack of temporal separation reported for four *Ctenus* species from central Amazonia (Gasnier 1996). Schmitt et al. (1990) found constant activity of *C. coccineus* over the whole night and a high activity of *C. getazi* during the first half of the night. Although nightly movements of individuals were not included in the scope of this study, no species-specific hunting times could be detected in the field. In tropical regions with weakly pronounced seasons as in RBT, the abundance of subadult and adult spiders is relatively low (Russell-Smith & Stork 1995; Silva 1996; Rego et al. 2005). The relatively low abundance of potential intraguild competitors, together with the spatial segregation of the speeies, makes it plausible that no species-specific nighttime preferences have developed. Considering the spiders' low-cost sit-and-wait predatory strategy, no significant saving of energy eould be achieved by hunting for only part of the night, reducing potential hunting success even more. Temperature near the spiders does not seem to be an important variable for hunting microhabitat

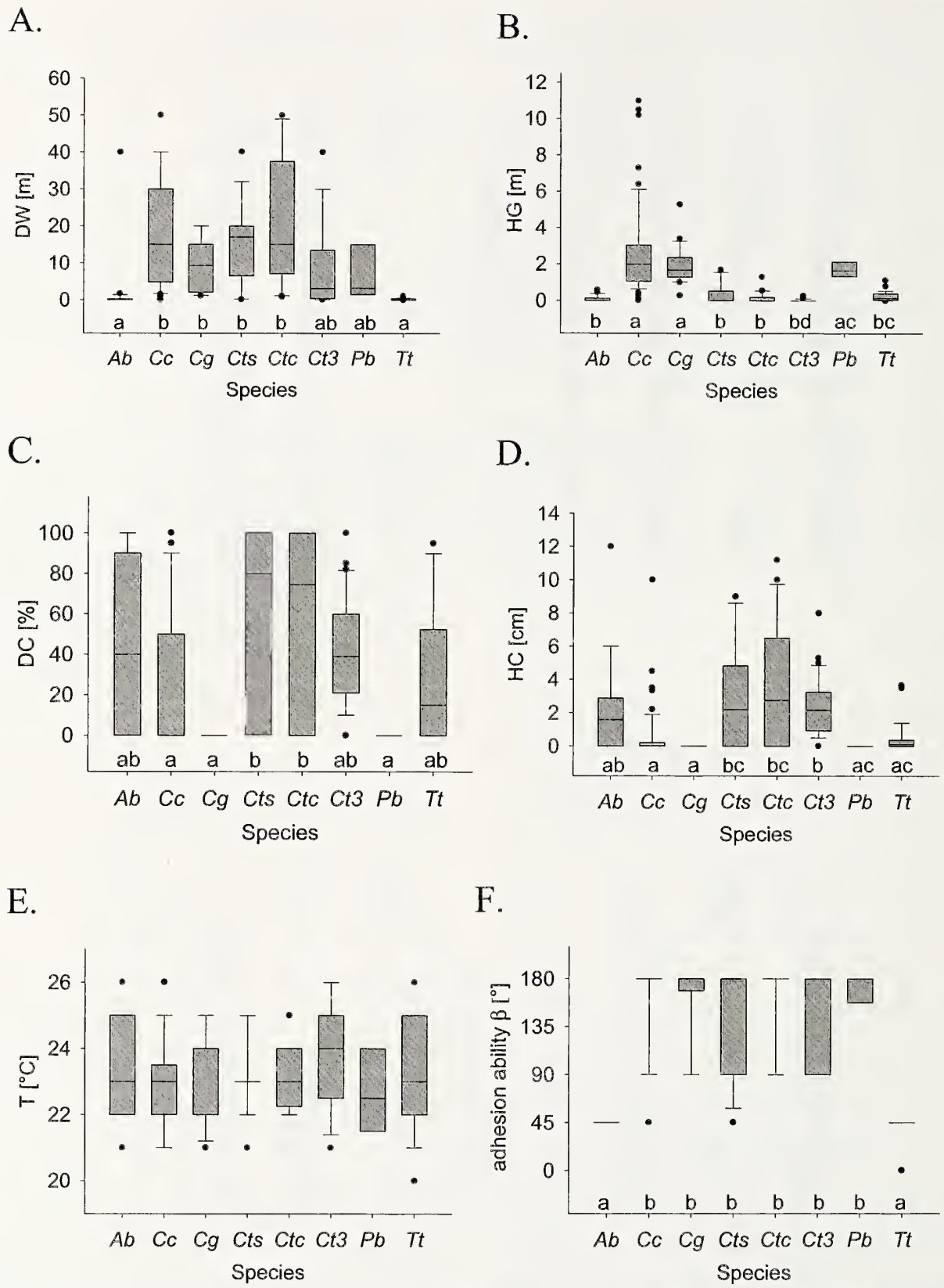

Figure 4.—Comparison of variables of the microhabitats and adhesion ability of the wandering spiders. Distance from the next water body (A), height above ground (B), degree of cover (C), height of cover (D), temperature (E), and achieved maximum angle of inclination in the adhesion tests (F). Different letters below box plots indicate significant differences based on Dunn's post hoc test. For species abbreviations see legend in Table 2.

choice, which probably mirrors the relative mieroclimatic homogeneity of the RBT forest.

Our findings on habitat separation are consistent with previously published results on vertical segregation in other generalized predators of arthropods, such as *Anolis* lizards (Reagan 1992), hylid frogs (Menin et al. 2005), and *Norops* lizards (D'Cruze & Stafford 2006). Among arthropods, generalist predators are mainly constrained by their tolerance for environmental conditions of the strata (Basset et al. 2003). In tropical Africa, most species of Ctenidae occurred on the forest floor and in the understory, with none in the canopy (> 3.0 m above ground) (Steyn et al. 2002; Sørensen 2003). In those forests, ctenids might be replaced in the higher forest strata by species of other wandering spider families. Unfortunately those studies were restrieted only to ctenids. In the Amazon, *Phoneutria reidyi* (F.O.P-Cambridge 1897) was also found on vegetation up to 5 m above ground (Torres-Sánchez 2000; Torres-Sánchez & Gasnier 2010). The total height range used by the RBT wandering spiders, especially *C. coccineus*, was probably underestimated because most data could only be obtained by observation from the ground. Using adequate canopy aeeess techniques should resolve that question. Our results indicate, however, that large ctenids frequently do occur at greater heights above ground.

The wide ranges in degree and height of cover in the microhabitats hunted by most of the species probably mainly reflects the conditions within the respective habitats. Thus, leaf litter appears to be an important factor for the three *Ctenus* species, but is less so for the other species. In other wandering spider assemblages, microhabitats may differ in depth or complexity of leaf litter (Uetz 1977; Gasnier 1996). The amount and complexity of leaf litter is considered to affect hunters as well as potential prey organisms by providing protection from different abiotic impacts, but also by offering shelter from predators (Fauth et al. 1989; Wise 1993).

We confirmed our hypothesis that the hunting microhabitat preferences of the sympatric wandering spider species would also be refleeted by their specific ability to adhere to smooth surfaces. Although adhesion abilities are probably of little importance in the semi-aquatic microhabitats of *T. tirimbina* and *A. bogotensis*, , this quality plays a more important role in vegetation dwellers. Due to their high adhesion ability, the three *Ctenus* spp. seem to be preadapted to a broad range of microhabitats, including the forest floor and also the higher vegetation. The high adhesion abilities of the vegetation dwellers (*Cupiennius* spp. and *P. boliviensis*) might be particularly advantageous when climbing on smooth surfaces, even on the undersides of leaves in head-down position.

Based on our results, the eight large wandering spider species of RBT can be assigned to three main subguilds:

1) **Semi-aquatic species** (sensu Graham et al. 2003): *Ancylometes bogotensis* and *Trechalea tirimbina* are strongly associated with water bodies and have poor adhesion abilities.
2) **Ground-dwelling species**: Three *Ctenus* spp. forage on the forest floor and hide among the debris and have good adhesive capabilities.
3) **Vegetation-dwelling species**: These species are strongly associated with vegetation and have very good adhesive capabilities: two *Cupiennius* speeies and *Phoneutria boliviensis*. *Cupiennius coccineus* prefers forest sites, while *C. getazi* and *P. boliviensis* are habitat generalists that may prefer treeless areas.

Our results on the wandering spiders agree with studies that show species-specific habitat preferences within different taxa of animals; e.g., assemblages of frogs, lizards, and spiders (Uetz 1977; Reagan 1992; Dias & Brescovit 2004; Menin et al. 2005; D'Cruze & Stafford 2006; Williams et al. 2006; Entling et al. 2007; Torres-Sánchez & Gasnier 2010).

The only site where large tropical wandering spiders have been studied in some detail is the Reserva Florestal Adolpho Ducke (RFAD) in central Amazonia, Brazil. *Ancylometes* species were frequently found on the ground near water (Azevedo 1999; Höfer & Brescovit 2000), *Ctenus* species lived mainly on the forest floor but also climbed into the lower vegetation stratum (Höfer et al. 1994; Gasnier 1996), and two *Phoneutria* species dwelled on the forest floor and on plants (Torres-Sánchez 2000; Simó & Brescovit 2001; Torres-Sánchez & Gasnier 2010). Habitat use within the local wandering spider assemblage therefore seems to be rather similar in RBT and in RFAD. The similarity of these two neotropical assemblages suggests that similar microhabitats and selection pressures have led to similar abilities and lifestyles of the species at both sites. Consequently, we expect similarity at the structural level, but not necessarily at the taxonomic level, among wandering spider assemblages of different geographical regions with similar climatic conditions.

In conclusion, the assemblage of sympatric wandering spiders at the Reserva Biológica Tirimbina showed a clear strueture, and the species differed clearly in habitat and hunting microhabitat selection. This points to the importance of habitat heterogeneity for species coexistence. These ecological preferences were correlated with abilities to adhere to certain microhabitat surfaces. Our data suggest the existence of assembly mechanisms for large hunting spiders that are based primarily on structural habitat parameters.

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Assessment of the probability of colonization of local spider communities in an experimental landscape

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Abstract. Establishment of communities is a dynamic process initiated by immigration. Therefore, movements of individuals within a metacommunity are important for maintaining and increasing species distribution. We present results of a small landscape-level experiment that manipulated habitat size and diversity. We examined the rates of colonization of spider species, and the richness, abundance and composition of foliage-dwelling spiders. Estimation of colonization rates was based on maximum likelihood. The experimental landscape was composed of five blocks with four patches (two large, 1 m^2; two small, 0.25 m^2). Less diverse patches had seedlings of one plant species, whereas more diverse patches had four species with diverse structures. Eight periodic censuses of spiders arriving in the patches were performed (average interval between censuses, 28 days). The initial composition of colonizers was significantly different from the final composition, but rates of colonization did not differ between sizes and diversities, or their interaction. Abundances of spiders were positively influenced by patch size. Compositions in each temporal sample were determined by differences in the species pool migrating and arriving at an experimental landscape irrespective of habitat size or diversity. Larger patches were more likely to receive more colonists representing a wider array of species than small patches. The probability of colonization was independent of patch size and diversity, which contradicts theoretical predictions. The results highlight the high colonization capacity of spiders on spatial and temporal scales.

Keywords: Community composition, immigration, passive sampling, temporal variation

The process of colonization includes survival and establishment, as well as immigration (Lomolino 1990). Immigration is the movement of organisms from the regional pool of species to a target habitat. It follows the phenologieal development of organisms in that habitat, which enhanees the probability of reproduction that would establish a population of a species. Then the population growth in the local community is determined by the set of available resources and the interactions with other species. There is a limit to population growth, and individuals exceeding the limit must either die or disperse to other habitats, restarting the colonization process. Colonization is a key process in the Theory of Island Biogeography (TIB) (MacArthur & Wilson 1967), which proposes that the number of species arriving on a given island should depend on the size of the island in question, as well as on its distanee from a mainland that serves as a species pool (Clark & Rosenzweig 1994). Larger islands receive more immigrants and, therefore, should show higher species richness. This is the definition of the area per se hypothesis for explaining the species-area relationship (Connor & McCoy 2001). Alternatively, the habitat diversity hypothesis postulates that larger areas may have more kinds of habitats, and hence more species, than small areas (Williams 1964; Nilsson et al. 1988). The TIB was appropriated in early years of fragmentation research to fit the patterns of richness observed in habitat islands, but ecological processes in remnants are in various ways critically influenced by direct interactions with surroundings, whereas such interactions are negligible on oceanic islands (Haila 2002). Ultimately, the matrix structure sets the pool of species that is able to act as immigrants.

In this paper, we present a small-scale landscape experiment that manipulated habitat size and diversity in a randomized block design. Our main goal was to examine whether rates of colonization of spider species are influeneed by the size and diversity of vegetation patches. To achieve this we employed a method based on maximum likelihood estimation to assess colonization rates from periodic surveys (Clark & Rosenzweig 1994). We assumed the presence of adult spiders in the patches as the final stage of a colonization event. Additionally, we assessed the patterns of abundance, richness, and composition of foliage-dwelling spider species in the vegetation patches. The experimental landscape was manipulated in such a manner that confounding variables supposed to influence the spider responses were controlled (Ewers & Didham 2006). Therefore, shape of patches, distance among patches and matrix surroundings were all held constant during the study period.

The experimental landscape was composed of five blocks with two levels of patch area: large and small; and two levels of patch diversity, more and less diverse. The comparison among these different patch treatments provides insight into the relative importance of size and diversity for spider colonization. When patch size is important independent of patch diversity, we may infer that the area per se hypothesis (Connor & McCoy 2001) is the main mechanism shaping the colonization; although the richness and abundance response to the size effect may reflect sampling artifacts like passive sampling of the regional pool of species (Schoereder et al. 2004). If patch diversity is important, independently of size, the probable important mechanism influencing colonization is habitat heterogeneity (Cramer & Willig 2005). If size and diversity both influence the eolonization rates in the same direction, the increase in pateh diversity is linked to increasing

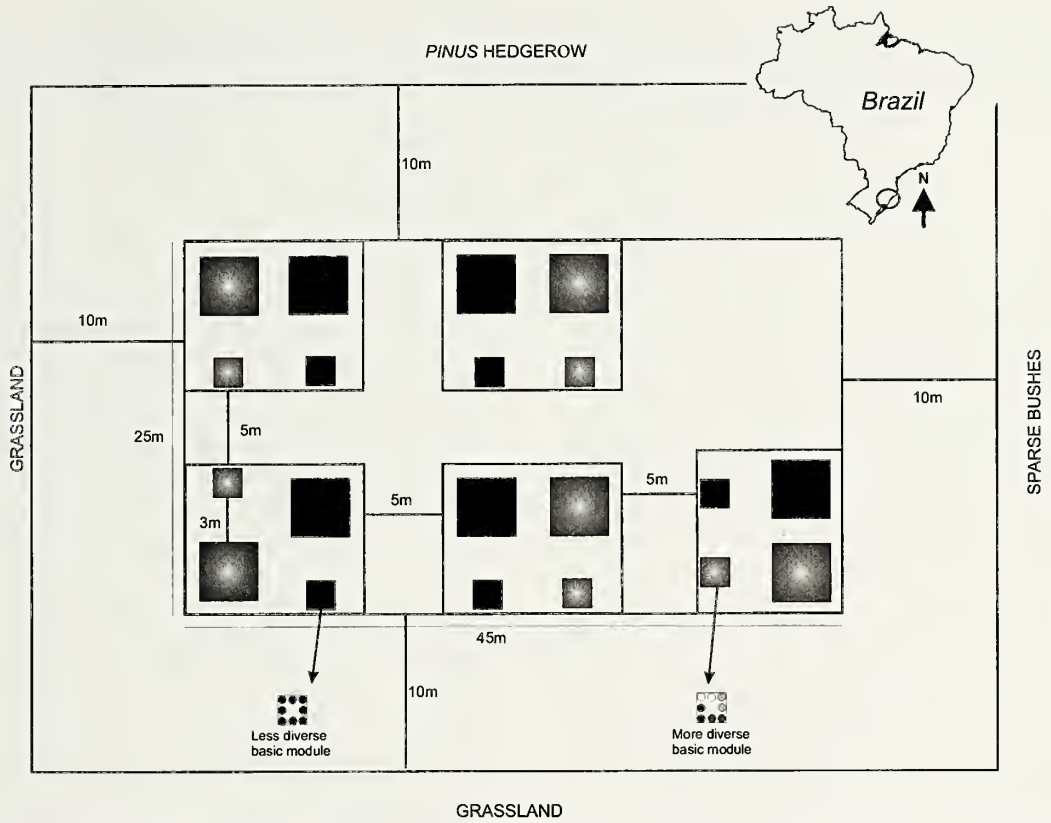

Figure 1.—Experimental landscape showing arrangements of the five blocks with the two levels of factors. Black squares = less diverse patches, shaded squares = more diverse patches. The matrix was set in a managed grassland. Factor levels were assigned randomly to each patch within a block. The less and more diverse basic modules were composed of the eight seedlings shown.

patch size. We expected an increase in colonization rates of spider species in larger and more diverse patches, based on the predictions of island biogeography theory and on the assumption that spider distribution and occurrence are strongly influenced by diversity in habitat structure (Uetz 1991; Wise 1993).

METHODS

Experimental design.—We conducted the study in an area of grassland on the coastal plain of southern Brazil (29°20′06″ S, 49°43′37″ W) (Fig. 1). The experimental landscape consisted of ca. 1125 m^2 of grassland surrounded by early successional vegetation (mainly sparse bushes) and a *Pinus* hedgerow (Fig. 1). In order to minimize the effect of the surroundings (nuisance factor) in the analyses, we utilized a randomized block design. The experiment was composed of two treatments (factors), area and diversity, randomly assigned to four experimental units in each one of five blocks. In ANOVA, inclusion of the block factor tends to improve power if the blocks are markedly more homogeneous than the whole area (Seltman 2012). In our case, the "whole" was the surroundings with different vegetation, which could influence the pool of species immigrating to the experimental units. Therefore, we had two treatments, area and diversity. Area had two levels: large or small, and diversity also had two levels: more or less diverse. The experimental unit was a plant patch that contained four size × diversity treatment combinations in each block. Therefore, we had 20 experimental units in the whole five-block experimental design. A two-factor fixed effects model was used to address 1) area main effects, 2) diversity main effects and 3) interaction between area and diversity. All factorial ANOVAs performed during the analyses used blocks of experimental units as the blocking factor.

Experimental units.—The experimental units were formed on a basic module of vegetation composed of eight seedlings. We used three native plant species [*Citharexylum montevidense* (Spreng.) Moldenke (Verbenaceae), *Eugenia uniflora* L. (Myrtaceae) and *Tabebuia chrysotricha* (Mart. ex A.DC.) Mattos (Bignoniaceae)] and one artificial seedling (Table 1). Each native seedling was rooted in a 2-l plastic pot. The artificial seedling was composed of three 20-cm-long wooden rods mimicking vegetation twigs, with five artificial leaves made of nonwoven tissue in each rod. The rods were partly buried in sand within the 2-l plastic pots. The basic module was thus composed of eight pots, with the seedlings situated in a 0.25 m^2 square (Fig. 1). The top of each seedling (leaf to leaf) was ca. 10 cm distant from the others. The basic module of the more diverse patches was composed of two pots of each natural seedling (six pots) and two pots of the artificial seedling, while the basic module of the less diverse patches was composed with only one previously randomly selected seedling (*E. uniflora*).

For the area treatment, the small patch level was simply the basic module patch (0.25 m^2), with either the more or the less diverse basic module, while the large patch level was composed of four basic modules distributed in a large square patch (1 m^2) (Fig. 1). We randomly assigned the position of each seedling

Table 1.—Mean ± standard deviation of the morphological structures from 10 randomly selected individuals of three plant species and one artificial seedling used in the vegetation patches. Artificial seedlings did not have branches, leaves were directly inserted in the artificial shoot.

	Shoot height	Internodes length (cm)	Number of branches	Branch length (cm)	Leaf length (cm)
Citharexyllum montevidense	77.3±5.4	11.2±1.1	6.6±0.7	5.4±0.5	7.7±0.3
Eugenia uniflora	43.4±3.1	16.6±1.9	9.3±1	3.2±0.3	3.9±0.3
Tabebuia chrysotricha	80.7±4.1	8.4±0.4	7.8±0.7	8.5±1	7.9±0.3
Artificial	30±0	3.7±0.1	0	0	4.4±0.1

group in each experimental unit, as well as the position of each experimental unit within each block.

Sampling procedure.—We removed the insects and spiders (defaunation) from seedlings prior to first spider collection by manual colleeting and by shaking the branches taking eare to not defoliate the seedlings. After the defaunation, we did eight periodic censuses during which we manually collected all spiders occurring in the experimental units. We spent one day sampling each block (four experimental units) so the whole census lasted five days in each period. The first sample was carried out in December 2010, thirty days after the defaunation. The mean days between two consecutive samples varied from 27.3 to 30.4 depending on the block. The spiders were collected in dry days, and the variation in the intervals of days between samples reflect rainy days that postponed the sample in that particular period. The month-long interval between censuses enabled us to analyze the first stage of community assembly: immigration and early establishment of adult spiders in the experimental units. All collected spiders were put in vials with alcohol, and the adult individuals were identified at species level whenever possible. Any adult male or female found in an experimental unit was considered a potential breeding individual. We utilized only adult spiders for the calculation of colonization rates and discarded the juveniles for two basic reasons. First, juvenile spiders are not suitable for identification at species level so they cannot be used to measure the colonization rate of species. Second, we found recently hatched spiderlings in various patches during the samplings, which could introduce bias in the analysis of abundance patterns. Voucher species are deposited in the spider collection of Museu de Ciências Naturais of Fundação Zoobotânica do Rio Grande do Sul, in Porto Alegre, Brazil.

Colonization rate measurement and analyses.—We utilized the method for estimating colonization rates from census data developed by Clark & Rosenzweig (1994). The method estimates the colonization rate for one species by first calculating parameter k, which equals the number of transitions from absence to presence of individuals in a patch during transitions of time, and parameter l, which equals the number of transitions from absences to absences. The first period (T_0) considered for the colonization rate calculation was the defaunation day. Therefore, there were eight time transitions from T_0 to T_8. For each experimental unit (vegetation patch) we computed k and l parameters for each species, based on the eight transitions. The colonization rate λ, where $\lambda = k\ /\ (k + l)$, is the probability that a species not present in the community will enter it in the time interval of approximately 28 days. In order to calculate the colonization rate of the entire community, we summed the k and l data for all speeies (Clark & Rosenzweig 1994) occurring in each experimental unit during the time periods and recalculated the colonization rate λ. Clearly, the k and l parameters are influenced by the frequency of oceurrence of each species and, consequently, by the abundances beeause more abundant species are likely to be more frequently found than rare ones. Nevertheless, k is positively related to the abundances, whereas l is negatively related to the abundances. In that sense, the denominator of the formula sets the weight of k and l to the measurement of colonization rate. If there are more transitions from absence to absence, the l parameter contributes more to the weight, while if there are more transitions from absence to presence, it is the k parameter that contributes more. Therefore, rare species (less frequent) contribute more with the l parameter, while dominant species (more frequent) contribute more with the k parameter. This characteristic prevents the bias that dominant/rare species may exert on the colonization rate estimate. This characteristic is apparent from the lack of influence of the adult spider abundances on the calculated colonization rates ($R^2 = 0.021$; $P = 0.55$). The influence of area, diversity and the interaction term on colonization rates was analyzed with a factorial ANOVA in the software Systat v. 11.00.01.

Composition analyses.—The variation in the composition of spider species over time was analyzed with a repeated measures two-factor permutational MANOVA, based on a chord distance dissimilarity matrix between experimental units. In that sense, we were able to assess possible interactions between the effeets of time and space on community composition. The abundance matrix was subjected to a log (x + 1) transformation in order to dampen the effects of dominant species. The repeated measures analyses were performed by restricting random allocations within the time periods. This approach allowed us to evaluate the variation in spider composition between the time periods, between the two factors, and the interaction terms (1000 permutations). We used the software Multiv v.2.63b to perform the analysis. It uses randomization tests based on resemblance measures between experimental units. The results are interpreted similarly to the ones in an analysis of varianee table. The method uses as the test criterion a sum of squares between groups of experimental units, as described by Pillar & Orlóci (1996).

Diversity analyses.—We tested the influence of size and diversity on adult spider abundance; i.e., the spiders that were identified at species level. In that sense, we considered adult abundance as a measure of successful colonization; i.e., potential breeding individuals that either mature or disperse as adults to the experimental units. Richness took into account the number of species found in each experimental unit. We utilized the adult abundances as a covariate in the species richness analysis in order to account for a sampling effect (passive sampling hypothesis). We pooled the periodic

measurements of adult abundanees and richness in order to perform two-factor ANOVAS. Analyses were performed in Systat 11.00.01.

RESULTS

We collected 2183 spiders belonging to 19 families. Fifty spider speeies were identified from 575 adults (Table 2). Web spiders were the most speciose and abundant spider group, with 33 species (66% of total richness) and 394 individuals (69% of total abundance). Among web spiders, the most speciose and abundant family was Theridiidae with 22 species and 345 individuals. The cursorial family, Salticidae, was the second most speciose family with seven species. The three most abundant web spider speeies were theridiids: *Cryptachaea hirta* (Taczanowski 1873) (n = 129), *Theridula gonygaster* (Simon 1873) (n = 109), and *Anelosimus ethicus* (Keyserling 1884) (n = 25). *Cheiracanthium inclusum* (Hentz 1847), the only Miturgidae (cursorial) species was also abundant with 102 individuals.

We did not find significant effects of the interaction between area and diversity on spider community colonization rates (ANOVA, $F_{1,16}$ = 0.04, P = 0.83) and the main effects of pateh size (ANOVA, $F_{1,16}$ = 0.8, P = 0.38) and diversity (ANOVA, $F_{1,16}$ = 1.8, P = 0.2) were also non-significant. Most of species (86%) showed less than 50% colonization probability during the experiment. As expected, the four most abundant species showed 100% of establishment probability. However, *Eustala saga* (Keyserling 1893) (n = 9) also showed high colonization potential.

We did not find significant effects of the interaction between time and patch size (PERMANOVA, SS = 5.0, P = 0.22) or patch diversity (PERMANOVA, SS = 4.7, P = 0.37) on spider composition, but the interaetion was significantly different among time periods (PERMANOVA, SS = 9.2, P = 0.001). Therefore, the composition responded only to the temporal effect. The PCoA ordination (Fig. 2) shows the species with the highest correlation eoefficients with axes 1 and 2 based on chord distanee dissimilarity between time periods. There are clearly three distinct spider compositions corresponding to initial, intermediate and final sampling periods.

We did not find significant effects of the interaction between area and diversity on spider community abundances (ANOVA, $F_{1,16}$ = 0.4, P = 0.55). However, we found a significant effect of patch size on abundance (ANOVA, $F_{1,16}$ = 71.7, P < 0.001) (Fig. 3). Patch diversity did not affect spider abundances (ANOVA, $F_{1,16}$ = 0.9, P = 0.34). Regarding spider richness, we found no interaction between patch size and diversity (ANOVA, $F_{1,15}$ = 1.4, P = 0.26). The resulting model of spider richness responses showed a significant effect of the adult abundances (ANOVA, $F_{1,15}$ = 7.4, P = 0.016) (Fig. 4), but patch size (ANOVA, $F_{1,15}$ = 0.2, P = 0.64) and patch diversity (ANOVA, $F_{1,15}$ = 4.0, P = 0.06) did not influence mean richness.

DISCUSSION

We found that the rates of colonization of spider speeies in the experimental landscape were not affected by size or diversity of habitat patches, in spite of temporal changes in the composition of colonizers. We sampled the community at very short periods, which emphasized the capacity of spider community to initiate rapid succession after disturbances (Stefano et al. 2007; Fattorini & Borges 2011). The local community structure underwent continuous adjustment under the regime of continuous change in its composition (Loreau et al. 2001). However, the alterations in the regional community structure did not translate into changes in the likelihood of colonization.

Our experiment showed a continuous flux of immigrants on the landscape that was able to arrive at the vegetation patches, a pattern also found for a spider eommunity in a Brazilian cerrado experiment, where spiders were the only taxon that continuously colonized litter plots (Vasconcelos et al. 2009). Initial spider community composition was largely determined by temporal differences in the pool of species that arrived and established at the experimental landscape, irrespective of habitat size or diversity. These changes in the regional pool of species composition did not affect the likelihood of local colonization over timc; i.e., the process of local habitat colonization did not respond to changes in the structure of regional community. If the system was subjected to unpredictable spatial disturbances, temporal variation might constitute a major source of spatial patchiness, and species would differ in their responsiveness to temporal environmental variation (Wiens 1976). In the present experiment, we continuously disturbed the spider community structure, which placed it in the first stage of succession, with steady vegetation patch condition over time. In that sense, we reproduced the non-equilibrium approach to community structure, which states that community structure is primarily determined in a non-equilibrium fashion by the interactions of the heterogeneity of the physical environment (size and diversity factors), disturbance (spider census) and recruitment (colonization) (Reice 1994).

We found that adult abundance was the only community characteristic responsive to the imposed factors; it was higher in the larger patches. The result indicates that a positive relationship may be established between abundance and area during the early colonization of patches, matching the passive sampling hypothesis (Connor & McCoy 2001). The short time frame between conseeutive samples in the experiment allowed us to discard strong interspecific interaction effects in structuring the initial spider community. Hypotheses linked to equilibrium theory, like resource concentration (Root 1973), may be disregarded as an explanation for the pattern. Therefore, mechanisms linked to populations' changes in abundance must be considered to explain area effects. Hambäck and Göran (2005) emphasizcd the importance of the search mode of animals in the observed relationships between insect density and pateh size. In that sense, spider behavior may be equivalent to some contact searchers (Bukovinszky et al. 2005), which are essentially unable to identify suitable habitat before alighting on the substrate. Consequently, dispersers have little control over their ultimate destination, and the decision to stay or leave a substrate is based on tactile clues after landing. Therefore, larger patches may passively receive more immigrants. Additionally, abundance explained richness. In that sense, higher richness in larger patches was an artifact of sampling, which implies that the actual species richness is unaffected by size (Schoereder et al. 2004).

Other studies have also failed to find a significant relationship between plant diversity and arthropod abun-

Table 2.—List of spider species collected during the eight time periods (T1 to T8) of the experiment. *n* = abundance.

Species	T1	T2	T3	T4	T5	T6	T7	T8	*n*
Anyphaenidae									
Tasata sp.	1	0	0	0	0	0	1	0	2
Teudis sp.	3	9	0	0	0	0	0	0	12
Araneidae									
Araneus unanimus (Keyserling 1879)	1	0	0	0	0	0	0	0	1
Argiope argentata Tanikawa & Ono 1993	0	0	0	0	1	0	0	0	1
Bertrana rufostriata Simon 1893	0	0	0	2	1	1	0	0	4
Eustala saga (Keyserling 1893)	3	2	1	1	0	1	0	1	9
Eustala albiventer (Keyserling 1884)	0	0	0	0	0	0	0	1	1
Corinnidae									
Meriola cetiformis (Strand 1908)	0	0	1	0	0	0	0	0	1
Deinopidae									
Deinopis anica Schiapelli & Gerschman 1957	0	0	0	0	0	0	1	0	1
Linyphiidae									
Anodoration claviferum Millidge 1991	0	2	1	0	0	0	0	0	3
Lygarina silvicola Millidge 1991	0	0	0	2	1	0	3	9	15
Lygarina sp.	1	4	0	0	0	0	0	0	5
Sphecozone rubescens O. P.-Cambridge 1870	0	5	2	0	0	0	1	0	8
Triplogyna ignitula (Keyserling 1886)	0	0	0	0	0	0	0	1	1
Miturgidae									
Cheiracanthium inclusum (Hentz 1847)	18	10	9	8	32	12	3	10	102
Oxyopidae									
Oxyopes salticus Hentz 1845	0	1	4	5	6	3	1	0	20
Philodromidae									
Berlandiella magna Mello-Leitão 1929	4	1	0	0	0	0	0	0	5
Salticidae									
Aphirape uncifera (Tullgren 1905)	0	2	0	0	0	2	2	0	6
Mopiopia labyrinthea (Mello-Leitão 1947)	0	0	1	4	4	1	0	0	10
Salticidae sp.1	0	0	2	2	1	1	0	0	6
Salticidae sp.2	0	0	1	1	1	0	0	0	3
Salticidae sp.3	0	1	1	1	1	0	0	0	4
Salticidae sp.4	0	0	0	0	2	0	0	0	2
Tariona sp.	0	1	0	0	0	0	0	0	1
Scytodiidae									
Scytodes inubituba Rheims & Brescovit 2009	0	1	1	1	1	0	0	0	4
Theridiidae									
Anelosimus ethicus (Keyserling 1884)	8	8	1	1	2	3	0	2	25
Chrysso pulcherrima (Mello-Leitão 1917)	0	0	0	2	1	1	1	0	5
Cryptachaea hirta (Taczanowski 1873)	18	21	19	21	21	15	5	9	129
Cryptachaea passiva (Keyserling 1891)	0	0	0	1	1	0	0	0	2
Cryptachaea pinguis (Keyserling 1886)	3	1	2	0	3	1	0	0	10
Cryptachaea rioensis (Levi 1963)	3	4	0	0	1	0	0	0	8
Cryptachaea sicki (Levi 1963)	2	0	1	0	0	0	0	0	3
Dipoena sp.	1	0	1	0	0	0	0	0	2
Exalbidion sp.	0	0	0	1	0	0	0	0	1
Neospintharus rioensis (Exline & Levi 1962)	0	0	1	0	2	0	0	0	3
Parasteatoda tesselata (Keyserling 1884)	0	1	0	0	0	0	0	0	1
Phycosoma altum (Keyserling 1886)	0	0	0	1	2	0	4	6	13
Steatoda iheringi (Keyserling 1886)	0	2	0	0	0	0	0	0	2
Theridion bisignatum (Mello-Leitão 1945)	3	6	3	0	2	1	0	0	15
Theridion pernambucum Levi 1963	1	0	0	0	0	0	0	0	1
Theridion plaumanni Levi 1963	2	1	0	0	0	1	1	4	9
Theridion sp.	0	0	0	0	0	1	0	1	2
Theridula gonygaster (Simon 1873)	4	8	10	16	26	34	10	1	109
Thwaitesia affinis O. P.-Cambridge 1882	1	0	1	0	0	0	0	0	2
Thymoites sp.1	0	0	0	0	0	1	0	0	1
Thymoites sp.2	0	0	0	0	0	0	0	1	1
Tidarren haemorrhoidale (Bertkau 1880)	0	1	0	0	0	0	0	0	1
Thomisidae									
Misumenops maculissparsus (Keyserling 1891)	0	0	0	1	0	0	0	0	1
Misumenops sp.	0	0	0	1	0	0	0	0	1
Thomisidae sp.1	1	0	0	0	0	0	0	0	1
TOTAL	78	92	63	72	112	79	33	46	575

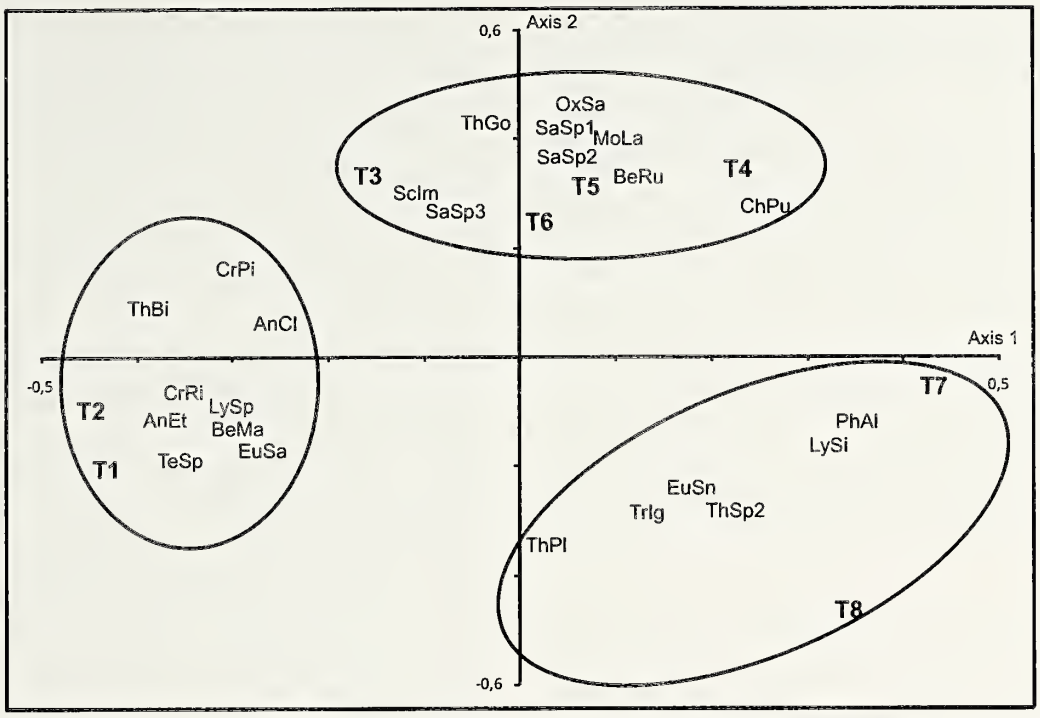

Figure 2.—PCoA ordination based on chord distance dissimilarity between the eight time periods. T1... T8 = time periods. Variation explained: axis 1 = 34.7%, axis 2 = 29.1%. Species showing the highest correlation coefficients with the axes: AnCl: *Anodoration claviferum*, AnEt: *Anelosimus ethicus*, BeMa: *Berlandiella magna*, BeRu: *Bertrana rufostriata*, ChPu: *Chrysso pulcherrima*, CrPi: *Cryptachaea pinguis*, CrRi: *Cryptachaea rioensis*, EuSa: *Eustala saga*, EuSn: *Eustala albiventer*, LySi: *Lygarina silvicola*, LySp: *Lygarina* sp., MoLa: *Mopiopia labyrinthea*, OxSa: *Oxyopes salticus*, PhAl: *Phycosoma alta*, SaSp1: Salticidade sp.1, SaSp2: Salticidae sp.2, SaSp3: Salticidae sp.3, ScLm: *Scytodes inbituba*, TeSp: *Teudis* sp., ThBi: *Theridion bisignatus*, ThGo: *Theridula gonygaster*, ThPl: *Theridion plaumanni*, ThSp2: *Thymoites* sp.2, TrIg: *Triplogyna ignitula*.

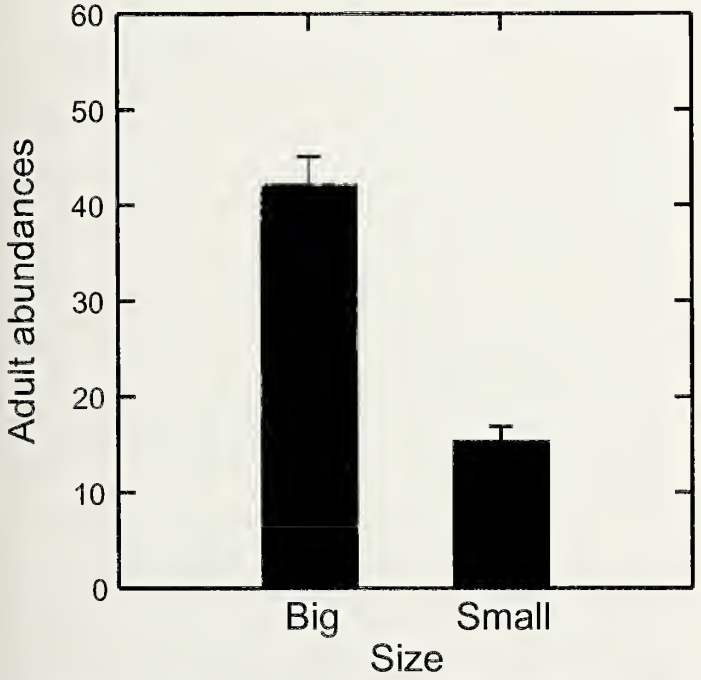

Figure 3.—Comparison of the means (± standard error) of adult abundance of spiders collected in large and small experimental patches of vegetation. Large patches = 1 m^2 (n = 10); small patches = 0.25 m^2 (n = 10).

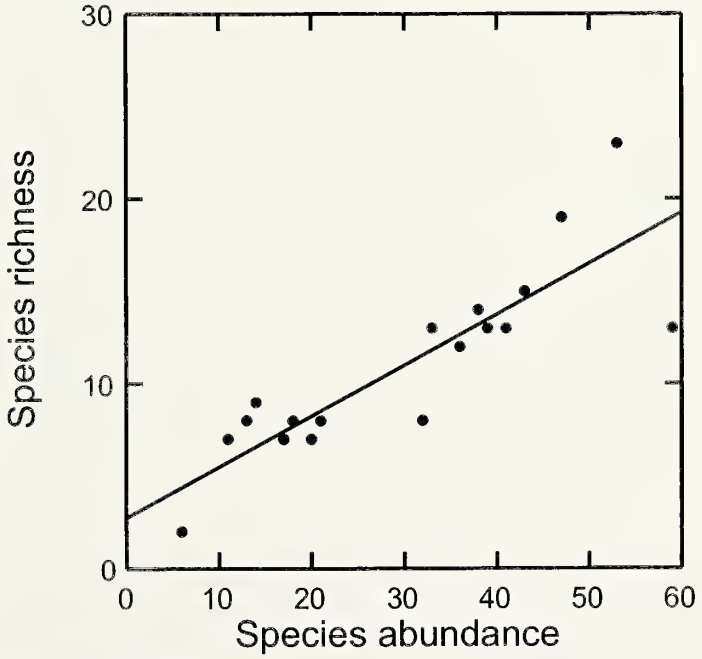

Figure 4.—Relationship between adult spider richness and abundance in 20 experimental vegetation patches (R^2 = 0.75, $P < 0.001$).

dance, but there was evidence of increased predator abundance in areas of high plant productivity and low diversity (Siemann 1998; Koricheva et al. 2000; Perner et al. 2005). Possible effects linked to prey abundance seem to be unimportant in shaping spider abundance in the experiment because there was not enough time between consecutive samples to allow important interactions between the spider community and other arthropods. Therefore, the equal abundance and richness of adult spiders in more and less diverse patches can be a response of the spider community to other aspects of habitat structure. Because web spiders comprised 70% of immigrants, it may be that the plant habitat in less diverse patches provides particular structures to support web construction. However, because the experimental design did not randomize the less diverse patches among the four seedlings, the diversity effect may be confounded by the presence of a plant identification effect that had a disproportional beneficial impact on the presence of web building spiders.

In the manipulative experiment employed in this study, we were able to physically manipulate attributes of the landscape in a controlled manner, while varying only patch size and diversity. Therefore, possible confounding factors like patch isolation, shape, and matrix were controlled. Additionally, the short interval between two consecutive samples maintained the spider communities at initial succession stage. In the experimental fragmented landscape, the responses of the spider community contradicted theoretical predictions about the influence of size and diversity on colonization rates. The community structure of spiders in this particular fragmented landscape is determined mainly by the continuous colonization proeess of the regional pool of species that settles the initial stage of eommunity succession after disturbances. However, we must stress that spiders have a high dispersal ability and even at large landscapes spider metacommunities may be not limited by dispersal (Baldissera et al. 2012). The small spatial and temporal scales used in this study restrict generalizations about the influence of area and diversity on a spider community. Therefore, we suggest the use of the present experimental design for future studies focusing on longer eolonization periods and larger-scale plots in order to better understand the responses of spider communities in fragmented landscapes.

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Abundance and diversity of spiders (Araneae) in barley and young leys

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Abstract. The fauna of surface-active spiders was studied in 12 cereal fields, with two types of subcrop, and in four young (17 months old) perennial leys (grass/clover). The fields were located in the southeastern (A), central (B) and western (C) parts of Norway. In total, 3945 spiders were caught from May to September 2004, using pitfall traps. Linyphidae was the most numerous family, with *Erigone atra* Blackwall 1833 representing 56% of all trapped individuals. The total numbers of spider species and individuals were significantly higher in leys than in barley at sites where both crops were present (sites A and B), with on average 11 species and 93 specimens in barley, and 20 species and 393 specimens in leys. Thus, young perennial leys appeared to provide a better habitat for spiders than did cereal fields, as has previously been documented for older perennial leys. The use of multi-species crops instead of a single crop species undersown in cereals, tended to result in higher spider species diversity, but it did not influence the total number of specimens. An ordination (DCA) showed a clustering of the spider fauna from the same site, but no clear separation between main crop types (ley vs. barley) was apparent. The main crops, subcrops, and the surrounding environs of the cropped field seem to affect the diversity and abundance of spiders.

Keywords: Cereal, community structure, farming, meadow

High abundance and diversity of spiders is considered to be important in both conventional and organic cropping systems because of the predatory function of spiders (Nyffeler & Benz 1987; Alderweireldt 1994; Marc et al. 1999; Kuusk et al. 2008). In organic farming, one has to rely on natural enemies, such as spiders and predatory insects, for pest suppression because the use of insecticides is not permitted. All spiders are true predators and constitute a heterogeneous group in terms of their feeding strategies, size, activity patterns, and dispersal modes (Marc et al. 1999). The diversity of many predatory insects and spiders is under pressure in agricultural landscapes (Kålås et al. 2010). With regard to pest suppression and the maintenance of biodiversity, both the density and diversity of spider populations are of interest in agricultural fields. The spider fauna in agricultural areas has so far been little studied in Norway (Andersen 1990; Pommeresche 2002, 2004).

Field borders and perennial crops, such as leys and pastures, often host more species and a higher number of spiders than do annual crops and cereal fields (e.g., Huusela-Veistola 1998; Ratschker & Roth 2000; Pommeresche 2004; Schmidt & Tscharntke 2005; Batáry et al. 2012). This may be related to a greater structural heterogeneity of perennial crops and a lower frequency of destructive soil tillage operations. There is a positive relationship between the spider fauna and the complexity of the local habitat (Rypstra et al. 1999; Gravesen 2008). Sunderland and Samu (2000) concluded that structural heterogeneity within the crop was more favorable for spider density in the cropped field than was structural heterogeneity in adjacent habitats. They found that spiders tended to settle in non-cropped and/or intercropped strips rather than within the main crop, unless the latter was diverse in structure.

Grasses are commonly undersown in cereals in order to suppress weeds and as a catch crop to prevent leaching of nutrients after the annual crop has been harvested (Sturite et al. 2007). Legumes are often used as subcrops in organic systems in order to improve the nitrogen supply (e.g., Reynolds et al. 1994). Perennial leys are usually established by undersowing in barley in order to obtain a cash crop in the year of establishment. One might expect that increasing habitat heterogeneity, by using multi-species instead of single species subcrops in cereals, would result in increased spider density and diversity. However, to our knowledge this assumption is as yet untested.

Soil tillage and harvesting operations cause disturbance and thereby reduce spider populations (Thorbek & Bilde 2004; Öberg & Ekbom 2006). For instance, 26 d after tillage, 93% fewer spiders were found in plowed fields and 80% fewer in non-inversive, deeply loosened fields than in untilled fields (Thorbek & Bilde 2004). Many lycosids appeared to survive the soil cultivation at sowing (harrowing and drilling) of cereals, but few linyphiids did (Öberg & Ekbom 2006). All the same, the linyphiids re-established themselves, or even increased in number, within a few weeks of such cultivation. Many linyphiids found in cultivated fields are pioneer species with a high potential for aerial dispersal, but some [e.g., *Oedothorax apicatus* (Blackwall 1850)] may also disperse cursorially; i.e., by walking (Thomas & Jepson 1999; Lemke & Poehling 2002). The length of time since crop establishment is thus a factor to consider when evaluating spider communities in agricultural systems.

Spiders respond to diversification at both local and landscape levels. A heterogeneous landscape, including perennial crops and field borders, is an important stimulant to the immigration of spiders into newly established crops (Sunderland & Samu 2000; Öberg et al. 2007, 2008; Schmidt-Entling & Döbeli 2009). At a landscape level, perennial grassland and field borders increase the density of aerially dispersing linyphiids, whereas adjacent field margins have the most influence on the density of cursorial lycosids (Huusela-Veistola 1998; Öberg et al. 2008; Schmidt-Entling & Döbeli

Figure 1.—Map of Norway showing the three sites (black dots) where samples were collected. The broken line shows the Arctic Circle.

2009). Also, the species diversity in both families is influenced by the heterogeneity of both the landscape and the adjacent habitat (Bishop & Riechert 1990; Öberg et al. 2008; Schmidt-Entling & Döbeli 2009).

The present study explores the spider fauna in young perennial leys and spring barley fields with mono-species or multi-species subcrops. Our aim is to describe the community structure and diversity of spiders found among these young but structurally differing crops, one to two seasons after abrupt and radical disturbances caused by soil tillage.

METHODS

From May to September 2004 we sampled surface-active spiders from 12 fields with spring barley and four fields with first-year leys located in southeastern (site A), central (site B), and western (site C) Norway (Fig. 1, Table 1). The barley was subcropped with either a mixture of grasses and clovers or ryegrass alone, or else grown without any subcrop. The barley and all subcrops were sown in April/May 2004. The first-year leys were undersown in spring barley the previous spring (2003) and consisted of 80–90% (w/w) perennial grasses and 10–20% clover species. These leys were 17 months old at the end of the spider sampling period. Annual mean (1961–1990) precipitation and temperature were 600 mm and 3.6 °C at site A, 890 mm and 5.3 °C at site B, and 1160 mm and 5.6 °C at site C.

Site description.—*Site A:* The Apelsvoll cropping system experiment (Eltun 1994; Korsaeth 2008) comprised two replicates of six different four-year crop rotations/systems laid out in a randomized block design. Each system consisted of

Table 1.—Characteristics of fields at three sites (A, B and C) in Norway, where spiders were sampled from May to September 2004. Sampled crop (corresponding to "Field") is marked with bold letters. The main crop is mentioned first and the subcrop after the slash. All leys were mixtures of grasses and clovers.

Field	Cropping system	Sampled crops (in bold) within crop rotation	Tillage	Plant protection
A1a, A1b	Conventional arable	**Barley**- Potatoes- Wheat – Oats	Autumn plowing	Chemical
A2a, A2b	Conventional arable	**Barley/ryegrass**- Potatoes -Wheat/ryegrass - Oats/Italian ryegrass	Spring harrowing	Chemical
A3a, A3b	Organic arable	**Barley/ley**-1st yr. ley -Wheat/ryegrass – Oats + peas	Spring plowing	Harrowing
A4a, A4b	Organic dairy	Barley/ley - **1st yr. ley** - 2nd yr. ley - 3rd yr. ley	Spring plowing	Harrowing
B1a, B1b, B3a, B3b	Organic dairy	**Barley/ley (B1)** - **1st yr. ley (B3)** -2nd yr. ley – Oats+peas	Spring plowing	Harrowing
C1a, C1b	Organic dairy	**Barley/ryegrass** - 1st yr. ley - 2nd yr. ley.- 3rd yr. ley	Spring plowing	Harrowing
C2a, C1b	Organic dairy	**Barley/ley** - 1st yr. ley- 2nd yr. ley- 3rd yr. ley	Spring plowing	Harrowing

four 15 m × 30 m rotation units (fields). We sampled spiders from fields with spring barley (A1–A3) and ley (A4) (Table 1). The barley was managed either conventionally without subcrops (A1), conventionally with perennial ryegrass as a subcrop (A2), or organically, with a grass-clover mixture (ley) as a subcrop (A3). Just before sowing the barley (13 May 2004), mineral fertilizers were applied to crops A1 and A2. Pesticides (herbicides, fungicides and insecticide) were used in the two latter systems. Crop A3 did not receive any chemical plant protection or fertilizer. The barley was harvested on 5 September 2004. The ley was not fertilized, and it was harvested on 14 June and 18 August 2004. The surrounding habitats of the cropping system experiment constituted mainly other crops (potatoes and cereals), field borders and some woodland and gardens.

Site B: The Kvithamar cropping system (Bakken et al. 2006; Johansen et al. 2008) comprised two replicates (blocks) of a four-year crop rotation/system. Each of eight rotation units was 55 m × 25 m. We sampled spiders from two replicates of two crop types, one with undersown spring barley (B1) and the other with ley (B3) (Table 1). Cattle slurry was spread before sowing of the barley on 4 May 2004 in B1, and on 22 April 2004 in B3. The barley was harvested on 24 August and the leys on 15 June and 1 September the same year. The system was located in an agricultural landscape predominantly consisting of cereal fields and some perennial leys, with permanent field borders and small roads.

Site C: This organically managed dairy farm was cropped with spring barley in a 3–5 year rotation with perennial grass-clover leys. In addition, there were permanent pastures nearby. We sampled spiders in two replicated transects within fields of spring barley, subcropped with either annual ryegrass (C1) or a grass-clover mixture (C2) (Table 1). Cattle slurry was applied prior to sowing the barley (15 May 2004). Field C1 was about 300 m × 100 m and C2 was 80 m × 25 m. The surrounding habitats included perennial leys, field borders, trees, drystone walls, and roads.

Sampling and identification.—We measured the activity of surface-dwelling spider species by sampling spiders in pitfall traps. These consisted of plastic jars, 6.5 cm in diameter, filled one-third with 50% (vol.) propylene glycol. Some droplets of a liquid detergent were added to break the surface tension. Spiders were sampled in eight rotation types with two replicates of each (a and b). Five traps were placed 2 m apart in a row at the center of each of the 12 sampled fields at sites A and B. At site C, we placed two replicate sets of traps more than 25 m apart, one in the center (b) and one (a) a bit closer to the field border in the two fields.

Pitfall trapping is the most commonly used sampling method for spiders (Hänggi et al. 1995), although it samples mostly surface-active individuals (Tretzel 1955; Topping & Sunderland 1992; Southwood & Henderson 2000). Since ground-dwelling species are more numerous in cultivated fields than the web-building/foliage-dwelling species (Nyffeler & Benz 1987), pitfall traps seemed most practical for our purpose. We were aware that this created a bias in both the diversity and the density, and thus we refer to the data as surface-active specimens.

During the sampling periods in 2004 (25 May–2 Sept at site A, 19 May–2 Sept at site B and 26 May–4 Sept at site C), we emptied the traps three to four times. They were removed from the fields during management operations involving machinery, and put back shortly afterwards. We pooled the five traps within fields and over time. Hence, each of the 16 fields was represented by one sample. Only adult spiders were identified and counted. The identification keys of Roberts (1993a,b, 1995) and Almquist (2005) were used, and nomenclature and taxonomy were in accordance with Platnick (2012).

Statistics and data analysis.—We analyzed the number of sampled individuals per trap day found in each field using ANOVA for each site separately. Field type (four levels at site A, two levels at site B, and two levels at site C) was regarded as a fixed factor and replicate (block) as a random factor. We conducted multiple comparisons of (least-square) means according to a Tukey's test. The data for sites A and B together ($n = 12$), were also subjected to ANOVA, with crop (ley, barley) as the fixed factor and site (A, B) as the random factor. The procedure MIXED in the statistical package SAS (SAS Institute Inc. 1999) was used for all analyses. In all tests, significance was assumed at P-levels < 0.05. We applied the same models for square-root transformed data for the total number of species found in each field.

The total number of species was also analyzed in a GLMM, implemented using the lmer function in the lme4 package (Bates & Sarkar 2006) developed for R (R Development Core Team 2008). We included site and crop as nested random factors in the model and principal crop type (ley, barley) as the fixed factor. The fixed factor was tested by comparing models with likelihood ratio tests. Models were made sequentially and reduced by backward elimination of non-signifieant effects ($P < 0.05$) (Crawley 2007; Zuur 2009).

A Detrended Correspondence Analysis (DCA) was run with CANOCO for Windows 4.5 (ter Braak & Smilauer 2002). We made the analysis and ordination of both the spider communities and the eight most abundant species from a dataset that included the species and their abundance in each field, from the total trap period. Rare species were down-weighted. The ordination placed similar communities of spiders close together in the diagram, while those less similar in species composition and abundance were placed further from each other (Jongman et al. 1995).

RESULTS

We trapped and identified 3945 spiders to species at the three sites. Thirty-seven species belonged to the Linyphiidae, thirteen to the Lycosidae, and seven to other families (Table 2). The most numerous species in the dataset were agrobiont linyphiids, where *Erigone atra* Blackwall 1833 represented 56%, *Oedothorax* spp. 13%, *E. dentipalpis* (Wider 1834) 5% and *Bathyphantes gracilis* (Blackwall 1841) 5% of all the trapped individuals. Most of the individuals of the genus *Oedothorax* were *apicatus* (Blackwall 1850) and even fewer were *retusus* (Westering 1851). The most numerous lycosids were *Pardosa palustris* (Linnaeus 1758) 8% and *P. amentata* (Clerck 1757) 3%, representing 58% and 21% of the lycosids, respectively.

The number of spiders varied between sites and crops (Tables 2 and 3). The mean number of spiders was 93 (1.02 ind. trap-day^{-1}) in barley fields ($n = 8$) and 393 (4.22 ind. trap-day^{-1}) in ley fields ($n = 4$), averaged across sites A and B, where both crops were present. The corresponding numbers of

Table 2.—The species and number of spiders found in barley and ley fields, sampled with pitfall traps, from May to September 2004. Only species names of spiders with two or more individuals in the material are shown, whereas the totals (sum) include all data. The number of spiders from the two replicates (a and b) are shown as a sum. For details about the cropping systems and management, see Table 1.

	A1a/b	A2a/b	A3a/b	A4a/b	B1a/b	B3a/b	C1a/b	C2a/b	% tot.
Linyphidae									
Erigone atra Blackwall, 1833	16	22	43	217	121	436	605	739	56
Oedothorax spp. (*apicatus* > *retusus*)	42	50	162	168	30	71		1	13
Erigone dentipalpis (Wider, 1834)	1	1		18	6	15	64	108	5
Bathyphantes gracilis (Blackwall, 1841)	1		7	6	73	81	5	10	5
Meioneta rurestris (C. L. Koch, 1836)	8	20	11	24	5	13		1	2
Porrhomma spp.	1		2	1	18	9			1
Bathyphantes parvulus (Westring, 1851)					5	22	10	2	1
Savignia frontata Blackwall, 1833		2	2	13	1	13	2		1
Leptorhoptrum robustrum (Westring, 1851)			1		4	8			
Allomenga scopigera (Grube, 1859)	1		1				2	3	
Collinsia inerrans (O. P.-Cambridge, 1885)							4	4	
Diplostyla concolor (Wider, 1834)		4		1		1		1	
Meioneta affinis (Kulczyn'ski, 1898)				1		1	1	2	
Diplocephalus latifrons (O. P.-Cambridge, 1863)				2				2	
Gongylidiellum vivum (O. P.-Cambrigde, 1875)							1	2	
Silometopus elegans (O.P.-Cambridge, 1872)		1		1			1		
Centromerita bicolor (Blackwall, 1833)				3					
Dicymbium nigrum (Blackwall, 1834)				1		1			
Neriene clathrata (Sundevall, 1830)								2	
Bathyphantes nigrinus (Westring, 1851)								2	
Dicymbium tibiale (Blackwall, 1836)		1		1					
Erigonella hiemalis (Blackwall, 1841)				1				1	
Gongylidium rufipes (Linneaus, 1758)							1	1	
Erigone longipalpis (Sundevall, 1830)							1		
Sum Linyphidae	70	103	230	461	263	674	698	884	86
Lycosidae									
Pardosa palustris (Linnaeus, 1758)	8	6	9	234	8	38	4	2	8
Pardosa amentata (Clerck, 1757)	2	1	7	36	11	30	4	21	3
Pardosa fulvipes (Collett, 1876)	2		1	37					1
Pardosa pullata (Clerk, 1757)	1		2	7	2	14	1	2	1
Pardosa riparia (C. L. Koch, 1833)		1	1	11	1				
Trochosa ruricola (De Geer, 1778)	2		3	3					
Pardosa agrestis (Westring, 1861)					5	3			
Pardosa nigriceps (Thorell, 1856)					1	3			
Trochosa terricola Thorell, 1856							1	1	
Pardosa paludicola (Clerk, 1757)				2					
Sum Lycosidae	15	8	23	330	29	89	10	27	13
Other spider families									
Pachygnatha degeeri Sundevall, 1830				5		2	2	4	
Pachygnata clerki Sundevall, 1823				1	2	5			
Robertus neglectus (O. P.-Cambridge, 1871)			1	3	1				
Micaria nivosa L. Koch, 1866			2						
Sum individuals	85	111	256	801	295	770	711	916	
Numbef of trap days	85	85	85	84	105	105	94	94	

species were 11 and 20. When comparing all fields at sites A and B, those cropped with leys had significantly higher numbers of species (ANOVA, $F_{1,9} = 25.3$, $P < 0.001$), specimens (ANOVA, $F_{1,9} = 62.0$, $P < 0.001$), specimens of linyphiids (ANOVA, $F_{1,9} = 54.2$, $P < 0.001$) and specimens of lycosids (ANOVA, $F_{1,9} = 10.6$, $P = 0.010$), than had fields cropped with barley (Table 3). The analyses of total number of species performed by GLMM confirmed the significance of the differences between principal crop types (GLMM, $z = 3.3$, $P < 0.001$). The number of individuals in the barley fields at site C was high (4.9 ind. trap-day^{-1}), and comparable to the number trapped in first-year leys at the other sites (Table 3). Linyphidae was the most numerous family in all fields at all sites. The proportion of lycosids was higher overall in leys than in barley crops.

The most numerous species in the cereal fields at sites A and B were the ballooning linyphiids *E. atra* (both in A and B) and *Bathyphantes gracilis* (B) and the more cursorial *Oedothorax* spp. (A), whereas *E. atra* and *E. dentipalpis* dominated in the cereal fields at site C (Table 2). The most abundant species in the leys were *E. atra* and *Oedothorax* spp. Of the lycosids, *P. fulvipes* (Collett 1876) was found only at site A, in southeastern Norway, and mostly in leys. *Pardosa palustris* and *P. amentata* were found at all sites, with slightly higher numbers of individuals in the ley fields.

Table 3.—Number of individuals and spider species trapped in different crops at three sites (A, B and C). Means within sites marked with different letters were significantly different from each other ($P < 0.05$). The main crop is mentioned first and the subcrop after the slash. All leys were mixtures of grasses and clovers. See Table 1 for details on the management.

Crops within site	No. of individuals per trap-day			Total no. of species
	Lycosidae	Linyphiidae	Total	
A1, Barley	0.09	0.41a	0.50a	8.0a
A2, Barley/ ryegrass	0.05	0.61a	0.65a	9.5a
A3, Org. barley/ ley	0.14	1.35a	1.51a	12.5b
A4, 1st year org. ley	2.00	2.74b	4.77b	21.5c
Standard error (SE)	0.42	0.27	0.43	
B1, Org. barley/ ley	0.14	1.25	1.41a	14.0
B3, 1st year org. ley	0.42	3.21	3.67b	18.0
Standard error (SE)	0.18	0.17	0.18	
C1, Org. barley/ ryegrass	0.05a	3.71	3.78	14.0a
C2, Org. barley/ ley	0.14b	4.70	4.87	17.0b
Standard error (SE)	0.02	0.52	0.53	

At site A, the number of species was significantly higher in the organically managed barley undersown with a grass-elover mixture (A3) than in the conventionally managed barley with ryegrass subcrop (A2) (Tukey, $t_3 = -5.3$, $P = 0.038$) or in barley without any subcrop (A1) (Tukey, $t_3 = -8.3$, $P = 0.011$) (Table 3). At site C, a higher number of species (ANOVA, $F_{1,1} = 235.9$, $P = 0.041$) and more individuals of lycosids (ANOVA, $F_{1,1} = 285.8$, $P = 0.038$) were found in barley undersown with grass-clover ley (C2) than in barley undersown with ryegrass (C1) (Table 3).

The ordination (DCA) showed a gradient of fields (spider communities) along the first two DCA axes (Fig. 2). Communities from the same site appeared to cluster, with site A mostly having the lowest scores on both axes, site C having the highest scores, and site B being intermediate. No clear clustering appeared relating to crops or subcrops. The gradient along DCA axis 1 explained 42.2%, and the gradient along DCA axis 2 an additional 12.8% of the variation in the species data, giving a total of 55.0% explained variation. The ordination also showed where the species were most abundant (shown for the eight most abundant species, Fig. 2). The first axis appeared to be related to the presence of species that were predominantly site-specific. For example, *Oedothorax* spp. and *Meioneta rurestris* were found mainly at sites A and B (low score on DCA axis 1), whereas *Erigone dentipalis* was mainly found at site C (high score on DCA axis 1). The second axis appeared to be related to the variation in species abundance between fields. As an example, *P. palustris* had the highest score on axis 2 (Fig. 2) and also the highest coefficient of variation between fields (data not shown), whereas *Pardosa amentata*, *M. rurestis, and Oedothorax* spp. were more evenly distributed between fields (lower coefficients of variation) and scored lower on axis 2.

DISCUSSION

In this study, the most abundant species were the agrobiont linyphiids *E. atra*, *Oedothorax* spp., *E. dentipalpis*, and *B. gracilis*. These were also among the most abundant species in agroecosystems in several other European studies (Thorbek & Bilde 2004; Schmidt & Tscharntke 2005; Öberg et al. 2007). We found *O. apicatus* in both young perennial leys and in cereals. By contrast, Schmidt and Tscharntke (2005) reported that this species was more frequently found in cereal crops than in perennial grass fields, due to its cursorial dispersal and tolerance of sparsely vegetated soil. We found more *Pardosa amentata* and fewer *Tenuiphantes tenuis* (Blackwall 1852) than Schmidt & Tscharntke (2005) reported for German agroecosystems. In the German study, Schmidt & Tscharntke (2005) found significantly more *Pardosa palustris* and *Pardosa pullata* (Cleck 1757) in perennial systems than in annual crops and wheat.

Several studies have revealed higher numbers of specimens and/or species in perennial meadows and leys than in cereal fields (e.g., Schmidt & Tscharntke 2005; Batáry et al. 2012). After collecting 4700 spiders, Schmidt & Tscharntke (2005) recorded forty-seven species in annual crops ($n = 26$), mainly wheat fields, and eighty in perennial grassland ($n = 16$). This indicated much higher numbers of species than we found, due to a variety of factors, but clearly indicating a higher number of species in older perennial systems. Our study shows that this divergence was also clear in relatively young leys (17 months old). Moreover, our results demonstrate a positive effect of ley on the spider fauna even in small experimental fields, where one may assume that the surrounding environment plays a relatively large role.

The inclusion of undersown crops in the barley, which probably made the spatial structure more similar to that in the ley fields, appeared to be an insufficient means of creating a spider habitat comparable to that of ley. So what can explain the favorable effect of ley on spider abundance and diversity? We believe that one reason is the longer length of time that has passed since plowing (one season) in the case of ley. Plowing has been found to be more harmful to spiders than has grass cutting (Thorbek & Bilde 2004).

Another factor, which may favor ley as a spider habitat, irrespeetive of its age, may be its relatively rich invertebrate fauna on which spiders feed. Spider abundance and diversity are influenced by the prey fauna, both at ground level and within the vegetation, which is thus of great importance for spider competitiveness. Collembolans and dipterans are especially important for the recruitment of juvenile spiders (Toft 2005; Gravesen 2008) and, along with aphids, they are also an important food source for adult spiders (Nyffeler & Benz 1988; Alderweireldt 1994). Collembola density and

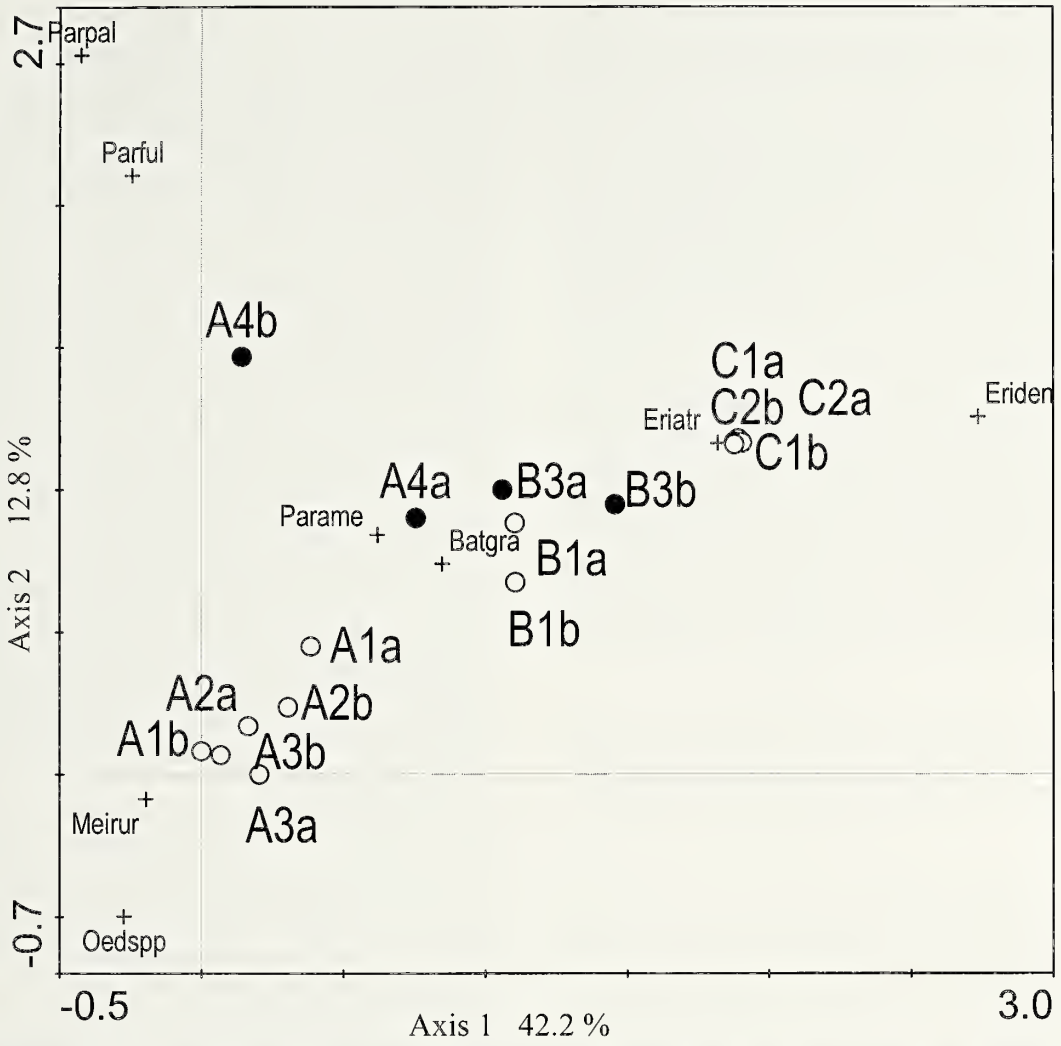

Figure 2.—Ordination (DCA) of the spider communities in different fields, based on species composition and density. Rare species were down-weighted. Black circles are spider communities from leys; open circles are spider communities found in spring barley. The ordinations of the eight most abundant species are shown with + and abbreviated name (Batgra = *Bathyphantes gracilis*, Eriatr = *Erigona atra*, Eriden = *Erigone dentipalpis*, Oedspp = *Oedothorax* spp., Meirur = *Meioneta rurestris*, Parame = *Pardosa amentata*, Parful = *Pardosa fulvipes*, Parpal = *Pardosa palustris*).

diversity have been found to increase with plant species richness in grassland (Sabals et al. 2011) and to respond positively to the use of liquid animal manure (Sokolowska & Seniczak 2005).

When looking solely at barley fields, the outcome at site C showed that a subcrop consisting of several species (ley mixture) resulted in a higher number of spider species than a single-species subcrop (ryegrass). This may be explained by the positive effects of increased spatial structure and prey fauna, as discussed above. The findings at site A were similar, as there were significantly more species in fields with multi-species subcrops (A3) than in fields with ryegrass as the only subcrop (A2). At site A, however, the difference may have been caused by a difference in pesticide use, rather than subcrop, since the management between A2 and A3 was very different. Several studies have shown that the use of pesticides decreases the density and/or diversity of spiders, but exceptions have also been found (e.g., Andersen 1990; Stark et al. 1995; Marc et al. 1999; Huusela-Veistola 1998). Chemical treatments influence the spider fauna either directly or indirectly; for instance, by lowering the number of prey.

The DCA ordination accounted for about 55% of the variation in species composition and abundance and revealed a clustering of fields, depending on site but not on crop. That the clustering pattern was so clearly related to site, even if the dominant species were found at two or all of them, was interesting. It indicated that factors outside the fields themselves had been important in forming the spider communities studied.

Considering the relatively high severity and frequency of disturbance in these crops, it is not surprising that the main factors governing the community structure appeared to be in the surrounding environments of the cropped fields on each site. An example reflecting this may be the high numbers of *E. atra* and *E. dentipalpis* in the cereal fields at site C (Table 2), where the surrounding leys must have influenced the fauna. The high proportion of perennial ley in the crop rotation, in addition to leys and pasture in the adjacent area, may have

been favorable for the *Erigone* species, thereby also increasing their number in the cereal fields. *Erigone* spp. can alternate between using and not using webs for capturing prey (Alderweireldt 1994), a strategy that is more successful in leys where cutting and grazing may favor species that have various prey strategies. Both species are found in perennial and annual crops, but often at a higher density in perennial crops (Schmidt & Tscharntke 2005). It is commonly reported that surrounding habitats and a heterogeneous landscape, including perennial crops, are important sources of aerial immigration to newly planted crops (e.g., Sunderland & Samu 2000; Öberg et al. 2007).

In summary, this study indicates that even very young perennial leys (17 months) constitute a better habitat for spiders than do undersown cereal fields. The use of multispecies crops undersown in cereals tends to result in a greater diversity of spiders than when the undersown crop consists of only one species. The clustering of sites, rather than of crops, in the ordination of the spider communities, confirms that factors outside the agricultural fields influence the spider fauna. Crops, subcrops, and the surrounding environs all seem to affect the diversity and density of spiders in cropped fields.

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An identification key for eggs and egg sacs of spiders of potential agroeconomic importance: a feasibility study

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Abstract. Information regarding the eggs and egg sacs of spiders found in agricultural crops in the San Joaquin Valley of California's Central Valley is presented as a feasibility study to aid inspection of international commerce. Egg diameter showed little variation within a species and strong variation among species; hence, it is a valuable diagnostic feature. Egg quantity per sac and sac dimensions showed greater and overlapping variation, yet are still somewhat diagnostic depending on the species. Least diagnostic was the phenology of egg sac production, but this characteristic was still useful in determining that some species finish producing egg sacs prior to crop harvest, indicating that they would not be found in transported produce. A diagnostic key utilizing the most useful of these features is provided. Overall, it appears likely that if keys regarding spider eggs and egg sacs could be developed, they could provide useful information in a real world economic situation.

Keywords: Araneae, agriculture, international trade

International commerce has led to the inadvertent redistribution of animals and plants throughout the world. Although many of these transplanted organisms probably die without establishing a viable population, many well-known examples exist of colonization by non-native organisms with detrimental economic effect (Jenkins 1996) or the displacement of native species leading to global species homogenization (Vander Zanden 2005). This process has been exacerbated by faster shipping methods, which for spiders increases the chance of surviving a journey to a foreign port (Kobelt & Nentwig 2008). Considering some of the detrimental animals on the invasive species list, spiders pale in comparison with other creatures that negatively impact commerce. However, concern still exists when toxic species such as those of the genera *Latrodectus* or *Phoneutria* are transported (Ross 1988; Reed & Newland 2002; Craemer 2006; van Meurs 2006; Vetter & Hillebrecht 2008).

Accurate identification is a priority. The misidentification of the harmless ctenid spider *Cupiennius chiapanensis* Medina Soriano 2006 as a potentially dangerous confamilial *Phoneutria* spider delayed the unloading of 960 cases of bananas in Texas (market value: $26,400 USD) (R.S. Vetter unpublished data). In a second incident, a shipping firm was almost required to fumigate 20 truckloads of Mexican wicker furniture and develop a personnel protection program for their employees due to a misidentification involving the same two ctenid species (Vetter & Hillebrecht 2008). Hence, misidentification of spiders can lead to economic loss in international trade due to greater expenditure in protection, use of pesticide control measures, or delays in the unloading of goods, especially perishable produce.

As much as spiders may cause consternation in international shipping, of equally great vexation for cargo inspectors is the discovery of a spider egg sac in goods (Reed & Newland 2002; van Meurs 2006). Delays due to waiting on spider emergence can last several weeks before identification is possible, risking spoilage of perishable cargo. As one can imagine, identification of newly emerged spiderlings could be a daunting task, especially for someone lacking extensive arachnological skill, even if the person is familiar with the local spider fauna. This difficulty is exacerbated when the person attempting the identification is in an international port and may not have extensive taxonomic knowledge or literature of the spider fauna of the country of origin, if that literature even exists.

Because of this situation, keys for spider eggs and egg sacs could benefit inspectors of American cargo being readied for international shipment. As we were not aware of any such information in the open scientific literature, we undertook this study as a heuristic device to examine the feasibility of such a novel project. Our hope is that by documenting the feasibility of this task, it might lead to more research on this understudied topic.

METHODS

We collected gravid spiders or egg sacs with guarding females from March through September in 2011 and 2012 in commercial crops of apples, pomegranates, grapes, citrus, and pears in central California's San Joaquin Valley in the following counties: Kern, Tulare, Fresno, and Madera. We also collected additional egg sacs of unique construction and unquestionable identity without females, based on the junior author's 24 years of experience in San Joaquin Valley agriculture. For some species, spiders were maintained to procure egg sacs; however, we typically only used sacs if they were laid in the first two weeks of captivity because we attempted to get natural fecundity measures, whereas artificial feeding of captive spiders might result in higher or lower quantities. The *Tivyna* spiders were an exception, in which five egg sacs were taken from two females over several weeks. The salticids construct egg sacs that are similar in form to one another but vary in size such that field-collected sacs required guarding females to be seen or collected simultaneously to assure species identification. When necessary, we collected and reared uncommon spiders, such as *Metacyrba* species, in Riverside until they produced egg sacs in captivity. We

generated most of the data for western black widow spiders from field-collected egg sacs in the Riverside area that were collected for a study of egg sac parasitism (Vetter et al. 2012a). We developed a phenology of oviposition for several species based on the junior author's 24 years of field notes.

We measured egg sacs with digital calipers for length and width, or diameter if spherical. Where the egg sac was ensconced within a retreat (*Cheiracanthium* and salticids), we measured the length and width of the retreat, as we surmised that an inspector would interpret the entire structure as an egg sac, not just the packet of eggs contained therein. We opened egg sacs and measured egg diameters with a Leiea MZ16 microscope fitted with an ocular micrometer. We measured the diameter for any one egg as the randomly-oriented subspherical egg lay across the micrometer instead of specifically trying to find the maximum or minimum size. The rationale behind this type of technique was to duplicate how an inspector would measure eggs in a rapid fashion, rather than taking the time to position each egg such that its greatest diameter would align with the micrometer. Nonetheless, most eggs were spherical. One exception, the araneid *Metepeira*, had bean-shaped eggs where we measured the greatest length.

We measured 10 egg sacs per species, with some exceptions, and 30 egg diameters, 10 from each of three egg sacs. We did not collect more of a particular species due to the time-intensive nature of the study and other limitations; we targeted 10 sacs as a feasible number to give us satisfactory measures of variation within the population, although often a few extra sacs were collected before we were assured that our target number had been reached. Also, an upper limit was necessary; otherwise the abundance of the common species would overwhelm our collecting efforts. For the less common species, we measured 15 eggs if possible in case we did not get sufficient quantities of eggs from additional specimens. Where clutches contained few viable-looking eggs or sacs contained damaged eggs, we measured these eggs as well as possible, often using more than three egg sacs. Obviously for those species with fewer than 10 eggs per sac, we used more than three sacs to obtain 30 measurements.

We counted the number of eggs or spiderlings per sac. If the sac contents consisted of eggs, the quantity was immediately counted. If the eggs had developed into embryos (rudimentary legs visible on the side of the egg) or beyond, we carefully closed the sac and counted the number of spiders upon emergence. Where spiderlings emerged and no additional live spiderlings were thought to be present, we treated the egg sac as follows to recover dead spiderlings or infertile eggs. We dipped the egg sac in 70% ethanol to reduce hydrophobicity and then placed it into a small plastic petri dish. A few drops of commercial bleach (6% sodium hypochlorite) were placed on top of the sac to dissolve the spider silk (Vetter et al. 1996). After most of the sac had been dissolved, we doused the sac with 70% ethanol in the petri dish to eliminate many of the visually disruptive bubbles that form as a result of the bleach action. We then examined the contents of the petri dish under a mieroscope whereupon shriveled, infertile eggs or dead spiderlings were removed and counted. These were added to the total of live spiderlings previously recovered to determine the total egg quantity of a particular sac.

Voucher specimens of the spider species are deposited in the California Academy of Sciences.

KEY TO EGG SACS OF SPIDERS FOUND IN SAN JOAQUIN VALLEY AGROECOSYSTEMS. COMMON SPECIES ARE IN **BOLD** TYPE, UNCOMMON SPECIES IN REGULAR TYPE. SPECIES WITH AN ASTERISK (*) ARE KNOWN TO OVIPOSIT IN THE HARVESTABLE PORTION OF AGRICULTURAL CROPS DURING THE HARVEST SEASON.

1a– sac suspended in web 2
1b– sac attached to substrate 8
2a– sac >10 mm 3
2b– sac <8 mm 4
3a– sac spherical or teardrop shaped with tightly woven exterior (Fig. 1), eggs free inside . . . **Latrodectus hesperus* Chamberlin & Ivie 1935
3b– eggs covered with loose silk (Fig. 2), eggs bound together in large connected matrix (Fig. 3) . . . ***Neoscona oaxacensis*** (Keyserling 1863)
4a– sac not spherical, somewhat angular (Fig. 4) or resembling a two-colored seed pod (Fig. 5) 5
4b– sac spherieal or subspherical 6
5a– sac somewhat angular, often triangular to pentagonal, suspended from silk at points with seam around perimeter (Fig. 4) . . . . . ***Dictyna calcarata* Banks 1904** or **Mallos pallidus* (Banks 1904) (in part)
5b– bicolored sac resembles a seed pod with an outer shell (Fig. 5), multiple sacs often strung together in succession (Fig. 6), eggs bean-shaped . . . . . *Metepeira arizonica* Chamberlin & Ivie 1942
6a– sac brown, eggs mottled tan and brown and visible through silk covering of few flimsy strands (Fig. 7), laid March through June . . . . . ***Theridion melanurum* Hahn 1831**
6b– sac tan 7
7a– sac never covered nor associated with dead plant material, laid April to September (Fig. 8) . . . . . ***Theridion dilutum* Levi 1957**
7b– sac may or may not be covered or adjacent to dead plant material, laid September to October (Fig. 9, 10) . . . . . *Tidarren haemorrhoidale* (Bertkau 1880) or ***Cryptachaea porteri* (Banks 1896)**
8a– sac dome-shaped with conspicuous flat brim around circumference, pure white silk of paper-like texture (Fig. 11) 9
8b– sac not papery, silk fibers apparent 10
9a– sac about 6 mm in diameter, eggs 0.76 mm in diameter . . . . . *Meriola decepta* (Banks 1895)
9b– sac about 8 mm in diameter, eggs 0.99 mm in diameter . . . . . ***Trachelas pacificus* Chamberlin & Ivie 1935**
10a– sac tiny (<1.8 mm), peaked dome shape, contains two to five eggs (Fig. 12) . . . . . *Tivyna moaba* (Ivie 1947)
10b– sac not tiny (>2 mm) 11
11a– sac <4 mm, covered with flimsy silk, eggs usually visible through silk 12

11b– sac >4 mm in at least one dimension 13
12a– sac about as high as wide, with some angular edges (Fig. 13) . . . . . ***Dictyna calcarata*** or **Mallos pallidus* (in part)
12b– sac flat, usually in a depression, eggs covered by a few flimsy silk strands (Fig. 14) . . . . . ***Oecobius navus*** **Blackwall 1859**
13a– sac often camouflaged more than 50% with detritus (Fig. 15, 16), February to May . . ***Hololena nedra*** **Chamberlin & Ivie 1942**
13b– sac covered 0% to 50% with detritus 14
14a– sac under bark, retreat of flocculent cribellate silk (Fig. 17) . . . . . *Kukulcania geophila* (Chamberlin & Ivie 1935)
14b– sac may be under bark or not, not surrounded by flocculent cribellate silk 15
15a– silk retreat variable in shape, fills space in which it is built, silk easy to separate with forceps, usually a retreat within folded leaves (Fig. 18) or under bark or between grape berries . . . . . ***Cheiracanthium mildei*** or ***C. inclusum***
15b– retreat-like sac, flat, longer than wide (Fig. 19), silk difficult to separate with forceps (salticids) 16
16a– sac small containing fewer than 20 eggs of less than 0.9 mm diameter . . . . . ***Sassacus vitis*** **(Cockerell 1895)** or ***Metacyrba taeniola similis*** **Banks 1904**
16b– sac large containing more than 20 eggs of greater than 1.15 mm diameter . . . . . *Thiodina hespera* Richman & Vetter 2004, *Phidippus audax* (Hentz 1845), or *Phidippus johnsoni* (Peckham & Peckham 1883)

RESULTS

Egg sacs.—The above key reflects the diversity of the egg sacs of the San Joaquin Valley spider fauna, including such traits as placement of the sac (suspended in the web or attached to a surface), overall shape (spherical, angular), degree of silk used to cover eggs, egg color, and egg sac silk color. Initially, we surmised that there would be a great deal of uniformity among the egg sacs; however, this was not the case. Some singular species were unique in their sacs, such as the seed-pod type sac of *Metepeira arizonica* (Fig. 5) or the massive sacs of the western black widow (Fig. 1) and *Neoscona oaxacensis* (Fig. 2, 3). On the other end of the spectrum, the large number of salticids with difficult-to-rip, retreat-like sacs required additional discriminatory skills to differentiate species. In regard to salticid sacs, pulling the sac with a pair of forceps in east-west directions, for example, opens up a hole. Subsequent pulling in a north-south direction opens up the silk, but closes the hole made in the east-west direction. Subsequent pulling repeats this process such that it required many such frustrating manipulations before the eggs were exposed, however, it did provide identification of the sac as that of a salticid spider.

Eggs.—The diversity of egg features provided sufficient numerical variability to allow for separation of species. Egg diameter is a life history trait with small variation within a species (Table 1). However, among the different species, egg diameter greatly varied from 0.39 ± 0.065 mm eggs of *Tivyna moaba* to the 1.24 ± 0.048 mm eggs of *Thiodina hespera*. However, we could discern with the unaided eye that egg diameter differences existed when two western black widow egg sacs were consecutively opened, measured, and the contents mixed in one petri dish. Therefore, we measured 50 western black widow eggs due to the greater variation.

Most eggs were glossy white or pearlescent, however, color differences provided useful diagnostic information for some species. The eggs of *Theridion melanurum* are mottled tan and brown and appear dark through the few silk strands holding them together.

Another variable that can be useful to exclude species from identification is the number of eggs per sac; however, this was more variable than egg diameter (Figs. 20 & 21). The smallest fecundity was recorded for *Tivyna moaba* with 3.6 eggs per sac (Fig. 20) ranging up to the most fecund spiders, *Neoscona oaxacensis* (mean = 230 eggs with a maximum of 622 in one sac) and the western black widow (mean = 283 with a maximum of 409 in one sac) (Fig. 21).

Seasonality of oviposition.—The seasonality of oviposition likewise provides characters useful in the key (Fig. 22). Some species (*Theridion melanurum* and *Hololena nedra*) are not a concern in harvested produce because they terminate oviposition prior to harvest season.

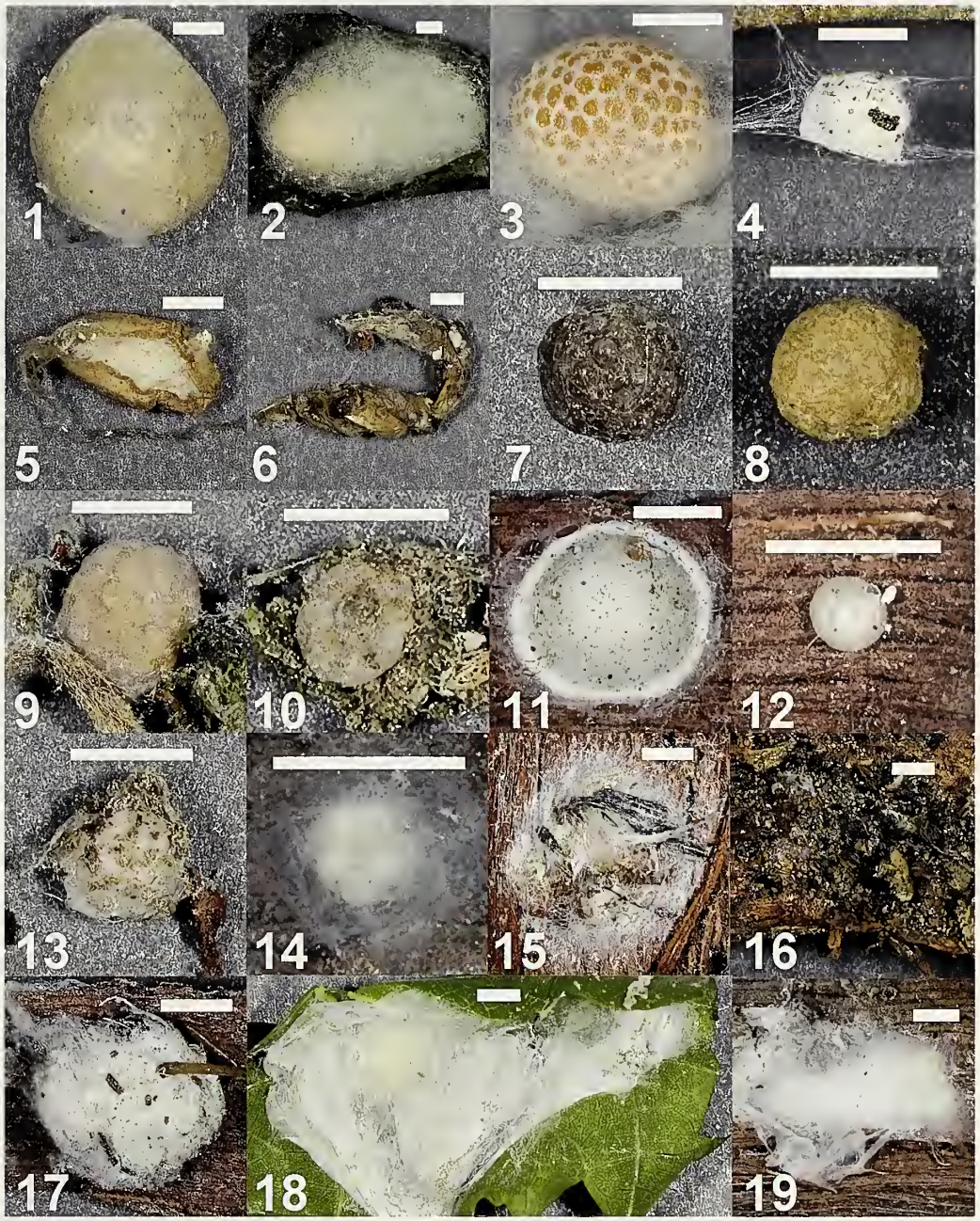
DISCUSSION

There are sufficient differences among spider egg sacs in morphology, egg diameter, number of eggs per sac and seasonal occurrence, that building a key and series of graphs and tables delineating these differences would be a useful and feasible project to identify spider species from a particular ecosystem. Some differences such as egg sac construction or egg shape could be diagnostic for the potential spider fauna of a region. However, one major caveat is that the spider fauna of the area of interest should be well documented prior to or generated during the course of the study. In this study, we included 22 species by searching several different crops via hand collection and based on the junior author's knowledge of the spider fauna of the area. In comparison, the studies of Costello and Daane (1995, 1997, 1998, 2003) involving at least four years of spider collecting from vineyards in several areas of the San Joaquin Valley with multiple sampling methods listed 29 species of spiders that potentially could be found.

Eggs diameters for most of the species that we examined had very little intraspecific variation (Table 1). Measuring a sample from a sac of unknown species should eliminate many species from consideration. Egg quantity per sac is obviously much more variable (Figs. 21 & 22) and hence, less diagnostic. However, it can be used to narrow the list of potential taxa. For example, an egg sac containing 30 eggs would surely exclude *Tivyna moaba, Oecobius navus, Mallos pallidus, Metacyrba taeniola similis* and *Sassacus vitis* as possible candidates.

Seasonality of egg sac production can also be an important factor. In the case of produce, the concern for egg sacs typically will be around the time of harvest, which for most commodities is summertime. Species that typically lay their eggs in the spring and terminate oviposition well before the harvest period such that they are unlikely to contaminate a cargo shipment can be removed as potential contaminants of shipped goods.

Location of oviposition also can be critical for assessing the value of a spider species as a potential pest in transported produce. For example, in vineyards, *Kukulcania geophila* only



Figures 1–19.—Egg sacs of spiders from San Joaquin Valley agricultural fields. Scale bar = 3 mm for all images. 1. *Latrodectus hesperus*. 2. *Neoscona oaxacensis*. 3. Exposed eggs of *Neoscona oaxacensis* in a connected matrix. 4. *Dictyna calcarata* sac when suspended in web. 5. Seed pod-like sac of *Metepeira arizonica*. 6. Several sacs of *Metepeira arizonica* strung together. 7. *Theridion melanurum*. 8. *Theridion dilutum*. 9. *Tidarren haemorrhoidale* sac with attached vegetation. 10. *Cryptachaea porteri* sac with attached vegetation. 11. *Trachelas pacificus*. 12. *Tivyna moaba*. 13. *Dictyna calcarata* sac when attached to substrate. 14. *Oecobius navus*. 15. *Hololena nedra* sac with about 50% detritus coverage. 16. *Hololena nedra* sac with about 100% detritus coverage. 17. *Kukulcania geophila* sac under bark. 18. *Cheiracanthium mildei* sac in the cavity of a curled leaf. 19. Sac of salticid spider.

deposits eggs under the bark of grape vines, so it could easily be excluded from the list of species contaminating shipments of produce. Additional biological knowledge may reduce concern over finding particular species in cargo. Although yellow sac spiders, *Cheiracanthium* spp., lay egg sacs in leaves of crops, the female guards the eggs. If a female is forced to abandon her egg sac or is killed, this would portend well for transported goods because *Cheiracanthium* spiderlings cannot emerge from the egg sac without the mother's help (Peck & Whitcomb 1970) and will die trapped inside.

Accurate identification of spiders should be critical for inspectors of imported cargo where the incorrect inclusion of a misidentified species might artificially and unnecessarily inflate the number of species of concern. Two examples, identified by New Zealand personnel, include *Latrodectus mactans* (Fabricius) 1775 and *L. geometricus* C.L. Koch 1941,

Table 1.—Egg diameters and egg sac dimensions for spiders found in San Joaquin Valley agroecosystems. Species are listed in increasing size of egg diameter. Sample size for eggs is 30 unless otherwise noted in parentheses next to the species name. The bean-shaped eggs of *Metepeira* are listed by greatest length only. Egg sac dimensions are presented for shortest and longest average ± SD measurement; if only one average is given, the egg sac is spherical or subspherical. Sample size for egg sacs follow egg sac dimensions in parentheses. Some egg sacs could not be measured as they were destroyed or misshapen during collecting and, therefore, sample sizes will not match in comparison to other portions of the study.

Species	Family	Egg diameter (mm)	Egg sac dimensions (mm)
Tivyna inoaba (5)	Dictynidae	0.389 ± 0.065	1.4 ± 0.4, 1.4 ± 0.4 (4)
Theridion melanurum	Theridiidae	0.539 ± 0.029	3.2 ± 0.6 (9)
Theridion dilutum	Theridiidae	0.555 ± 0.031	2.4 ± 0.4 (16)
Oecobius navus	Oecobiidae	0.555 ± 0.024	3.1 ± 0.5; 3.9 ± 0.5 (14)
Dictyna calcarata	Dictynidae	0.564 ± 0.024	3.3 ± 0.5; 3.8 ± 0.4 (10)
Tidarren haemorrhoidale	Theridiidae	0.566 ± 0.017	3.4 ± 0.6 (10)
Cryptachaea porteri	Theridiidae	0.594 ± 0.017	2.8 ± 0.6 (6)
Mallos pallidus	Dictynidae	0.614 ± 0.039	2.9 ± 0.8; 3.7 ± 0.7 (5)
Meriola decepta (4)	Corinnidae	0.764 ± 0.021	6.4; 6.4 (1)
Sassacus vitis	Salticidae	0.818 ± 0.029	7.0 ± 1.8; 13.5 ± 3.4 (11)
Metacyrba taeniola similis	Salticidae	0.868 ± 0.047	8.7 ± 1.9; 13.5 ± 5.0 (4)
Neoscona oaxacensis	Araneidae	0.950 ± 0.092	17.1 ± 1.0; 23.8 ± 6.0 (8)
Metepeira arizonica	Araneidae	0.958 ± 0.035	3.9 ± 0.6; 8.7 ± 1.5 (11)
Trachelas pacificus	Corinnidae	0.989 ± 0.042	7.7 ± 1.3; 8.4 ± 1.1 (12)
Latrodectus hesperus (50)	Theridiidae	1.044 ± 0.061	10.8 ± 1.5; 13.1 ± 2.2 (15)
Cheiracanthium mildei	Miturgidae	1.081 ± 0.064	17.5 ± 3.5; 25.0 ± 7.1 (2)
Hololena nedra	Agelenidae	1.135 ± 0.047	10.8 ± 1.5; 13.11 ± 2.2 (15)
Kukulcania geophila (10)	Filistatidae	1.141 ± 0.029	7.1; 8.1 (1)
Phidippus johnsoni	Salticidae	1.212 ± 0.075	24.3 ± 4.0; 40.0 ± 0.0 (3)
Phidippus audax (15)	Salticidae	1.216 ± 0.069	33.0, 33.0 (1)
Thiodina hespera (15)	Salticidae	1.243 ± 0.048	—

which were reported to have been intercepted from California produce (Reed & Newland 2002). This is highly unlikely. First, *L. mactans* is not found in the western United States and we know of no records of it ever being collected in California. We surmise that misidentification occurred because the diagnostic feature of *L. mactans* (a red dot anterior to the anal tubercle on the dorsal abdominal surface) was actually the rarely-occurring remnant juvenile coloration of *L. hesperus* (Kaston 1970, R.S.Vetter pers. observ.), the only black widow species in California. A similar misidentification as *L. mactans* was made for a *Latrodectus* spider (surely *L. hesperus*) found in southern California produce transported to Ireland (Ross 1988). Second, the pantropical *L. geometricus*, has only recently been found in California (Vincent et al. 2008). It was not known anywhere in California at the time of the report of Reed & Newland (2002) and, as of 2012, it has not been found in Central Valley agricultural areas. Additionally, it may never colonize agricultural areas because, in southern California, it is restricted almost entirely to urban locations (Vetter et al. 2012b). We speculate that this was probably

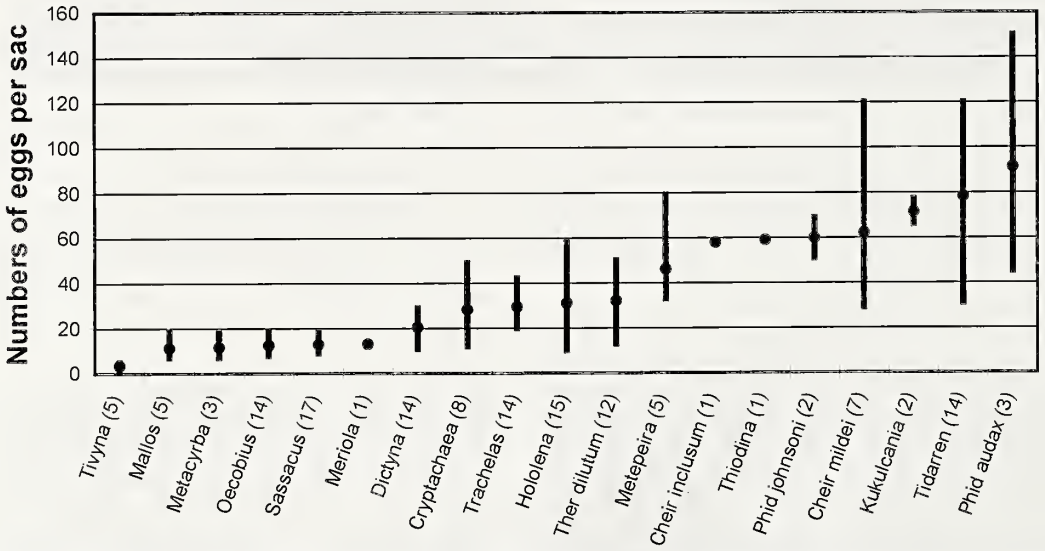


Figure 20.—Minimum, average and maximum eggs per sac for San Joaquin Valley agroecosystem spiders that lay less than 160 eggs per sac. The number in parentheses following the taxon name is the sample size.

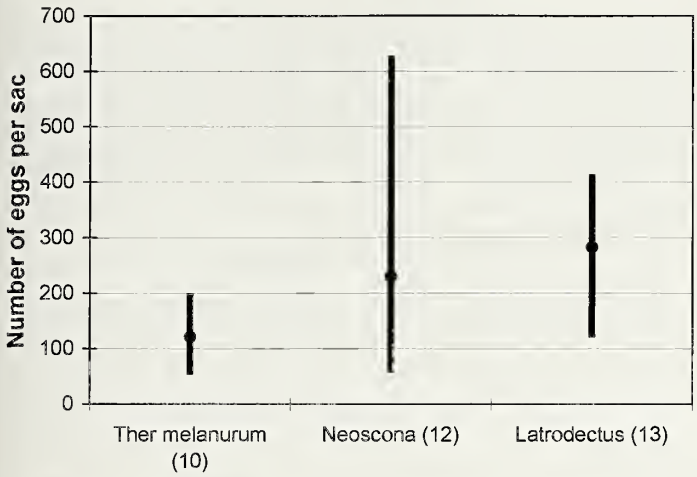


Figure 21.—Minimum, average and maximum eggs per sac for San Joaquin Valley agroecosystem spiders that can lay more than 160 eggs per sac. The number in parentheses following the taxon name is the sample size.

another misidentification of an immature *L. hesperus*, which has similarities in the striped pattern (Vetter 2012) and is frequently mistaken as *L. geometricus* by non-arachnologists (R.S.Vetter pers. observ.). Interestingly, although misidentifications have been made involving these three *Latrodectus* spiders, their egg sacs are easy to differentiate from one another: *L. hesperus* (yellowish tan, spherical or teardrop shaped), *L. mactans* (white, spherical or teardrop shaped), *L. geometricus* (tan, spherical and covered with silk spikes). Yet both non-Californian species were on New Zealand's list of concern for California trade (Reed & Newland 2002). We feel that with more information such as a key to eggs and egg sacs of spiders that these mistakes could be minimized or prevented.

Several limitations are evident in this study. First, a major caveat affecting the utility of the information presented here is that identification of a spider to species involving examination of an egg sac collected in the real world situation of a cargo hold requires that the sac sustains little damage during transport. This may be an overly optimistic supposition as sacs may get crushed during produce harvesting and processing or when cargo shifts during transport. Then again, if the sac is destroyed, it will not produce spiderlings to unleash into the new habitat, although the presence of a crushed egg sac may still raise concerns that there are more in the cargo undiscovered. One of us (R.S.Vetter) is currently undertaking a study regarding spiders found in international cargo brought into North America. On four occasions, egg sacs have been found on bananas, some with live spiderlings, including some of these discovered by the homeowner after taking fruit home (which, of course, caused considerable excited concern). Second, another aspect of elimination of species from consideration is that not every species in an agricultural area uses the harvestable portions of the plant for oviposition. This is positive from the standpoint that these spiders can be removed from the list of potentially imported

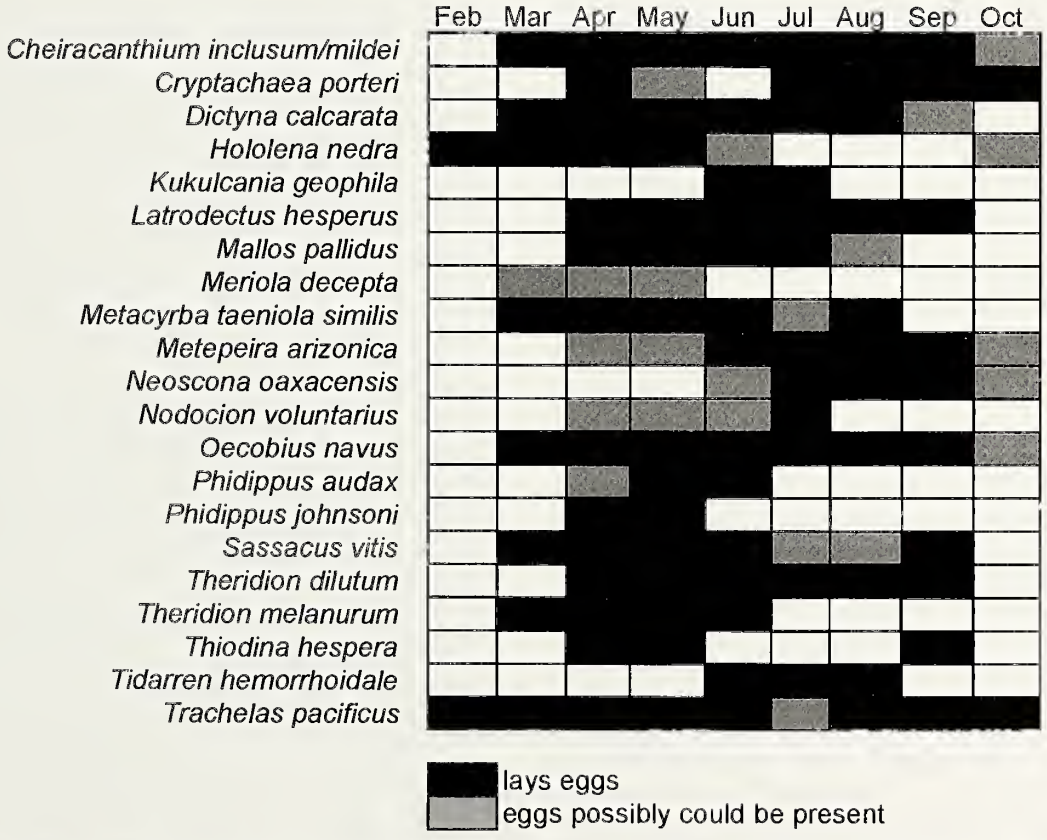


Figure 22.—Seasonality of egg laying for San Joaquin Valley agroecosystem spiders based on the 24-year experience of the junior author with months marked as eggs present (black) or eggs possibly present (gray). Some of these species listed here are absent from previous figures because their egg sacs were not detected during the 2-year course of this study.

egg sacs; however, it still does not allow sacs to be easily located in the field and measured so that information may be presented in order to exclude them more definitively from the list of potential transportable species. Although gnaphosid spiders were sufficiently common in San Joaquin Valley fields, their egg sacs were not discovered during the 2-year duration of this study. We also were not able to proeure egg sacs from female specimens that were collected. Possibly some species only hunt for food on the crop but lay their eggs under rocks or dirt clods or in places other than the fruit or vegetables that were being grown, or camouflage their sacs so well that they cannot be easily detected. Third, we had low sample sizes for some species because they were uncommon in the fields or they did not oviposit where their eggs could easily be found. A larger sample size for each species would provide more accurate information, especially for the number of eggs per egg sac. If a future project is deemed feasible, sufficient funding should override this deficiency. Fourth, we only looked at egg sacs and eggs; egg sacs do not always contain eggs. Some of the information provided here becomes superfluous once embryos develop and especially if the sac contains spiderlings. The degree of differentiation presented here could be further enhanced with additional life history characteristics such as description of the second instars. This would benefit the discrimination of the salticids in particular. For example, although *Sassacus vitis* and *Metacyrba taeniola similis* eggs and egg sacs are difficult to distinguish from one another, *Metacyrba* spiderlings emerging from the sac resemble the adults as almost exact miniatures such that they were recognizable to genus. Similarly, although *Thiodina hespera* and the two *Phidippus* species had equivalent-sized eggs and similar retreats, *T. hespera* spiderlings emerge as very pale individuals and have a partial complement of the diagnostic bulbous setae on the ventral surface of Tibia I, whereas *Phidippus* spiderlings are dark (R.S. Vetter, personal observation). Finally, DNA determination of adults might allow one to differentiate the difficult-to-separate species of egg saes.

To our knowledge, this is the first time that a diagnostic key for spider eggs and egg sacs for species separation has been generated in the open scientific literature. The only other readily accessible souree of information for diseerning spider egg sacs is a field guide to arthropod tracks and signs for the curious nature enthusiast which covers North America and has a modicum of information on spiders (Eiseman & Charney 2010). Although building a diagnostic key for eggs and egg sacs is a tedious and time consuming task, in some commercial venues there may be a need for developing such differentiation devices. The eventual determination of such a project will probably be driven by economie need and feasibility. Although this study was undertaken as a feasibility study, we feel that it is a good first effort and should offer some practical value for inspectors in central California's San Joaquin Valley.

We also hope that this study might spur other researchers to consider developing similar research along these lines because the documentation of different egg demographics might be an interesting and useful addition to the arachnological field in arenas of pure and applied research. One of the most useful functions of this study was to pare down the number of spiders from an overall species list to those that are candidates for ovipositing in harvestable crops. By providing information regarding seasonality and location of oviposition, the final number of species that might be found by inspectors in an agricultural crop is a small subset of the total potential species in the spider fauna of that geographic area, which should streamline identification efforts.

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Morphological phylogenetic analysis of the spider genus *Physocyclus* (Araneae: Pholcidae)

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Abstract. With 30 species and a natural distribution in North America, 28 confined to Mexico, *Physocyclus* Simon 1893 is the most diverse genus within the pholcid spider subfamily Arteminae. This paper provides the first phylogenetic test of the genus's monophyly through a cladistic analysis of 54 morphological characters using equal and implied weighting. The equally weighted analysis found 12 most parsimonious trees, whereas the analysis with implied weights varying the concavity values (K = 6–10) found five or six most parsimonious trees. The monophyly of the genus *Physocyclus* is supported by three synaphomorphies: 1) the paired ventral apophysis on the anterior part of the epigynum; 2) the lateral constraints in the middle part of the epigynum; and 3) the arc of the uterus, with a single sclerotized projection on the anterior part. The genus *Physocyclus* contains two clades treated as species groups: the *globosus* group, with 11 species, and the *dugesi* group with 19 species. The species relationships within the *globosus* group were better resolved than those in the *dugesi* group. The *globosus* group has a biogeographical distribution pattern in the Mesoamerican and Mexican Mountain biotic components, whereas the *dugesi* group has a distribution pattern in the Mesoamerican and Continental Nearctic biotic components. Given the complex biogeography in Mexico, apparently a large-scale vicariant event separated the two major clades within the genus *Physocyclus*.

Keywords: Cladistic analyses, morphology, species groups

The spider family Pholcidae ranks among the most diverse of web-building spider families, currently with 90 genera and 1330 species (Platnick 2013). These spiders are found in temperate, tropical and subtropical forests, with numerous synanthropic species and often in geographic areas and habitats that are severely threatened by human activity (Huber 2000, 2011b). In the New World, a great number of species remain unknown (Gertsch 1982; Huber 1997, 1998, 2000). Huber's (2000) paper is the most complete work for the New World pholcids, particularly from South America. For North Ameriea, including Mexico, the principal taxonomic contributions were made by Gertsch (1971, 1973, 1982), Gertsch and Davis (1937, 1942), and Gertsch and Mulaik (1940). Lately, the most recent important taxonomic contributions for North America were made by Slowik (2009) with the taxonomic revision of the genus *Psilochorus* Simon 1893 and Valdez-Mondragón (2010, 2013) with the taxonomic revisions of *Physocyclus* Simon 1893 and *Ixchela* Huber 2000, respectively. Huber (2000) described and redescribed some genera and new species from Mexico. The description of *Modisimus deltoroi* by Valdez-Mondragón and Francke (2009), and the taxonomic revisions of Valdez-Mondragón (2010, 2013), have been the latest taxonomic contributions made for Mexican pholcids. Currently, there has been considerable progress in the knowledge of pholcids from Mexico, with 13 genera and 162 species known.

Despite numerous recent revisions, the diversity of pholcid spiders in the New World is still inadequately known, and many species await description, mostly from previously underrepresented regions of Central and North America (Huber 2000). In Brazil, for example, no fewer than 39 new species were found in the Atlantic Forest (Huber & Rheims 2011), it being one of the most diverse areas in the country. Considerable fieldwork and research at biological collections where many specimens are deposited, not only from Mexico but also from the rest of Central and North America, are also necessary. For example, Valdez-Mondragón (2013) described 10 new species of the genus *Ixchela* Huber 2000 from Mexico and Honduras, and another five are currently being described (A. Valdez-Mondragón unpubl.).

Huber (2011a) divided the family Pholcidae into five subfamilies based on previous cladistic analyses of morphological and moleeular data and on qualitative character assessment (Huber 2000; Bruvo-Madarić et al. 2005; Astrin et al. 2006): Ninetinae Simon 1890, Arteminae Simon 1893, Modisiminae Simon 1893, Smeringopinae Simon 1893 and Pholcinae C.L. Koch 1850. Currently, the genus *Physocyclus* is placed within the subfamily Arteminae, which includes 77 species in five genera, with *Physocyclus* and *Trichocyclus* Simon 1908 being the most diverse genera, containing 30 and 23 species respectively (Valdez-Mondragón 2010; Huber 2011a; Platnick 2013). Phylogenetic evidence, both morphological and molecular, suggests a close relationship of *Physocyclus* with *Artema* and *Trichocyclus* (Bruvo-Madarić et al. 2005; Huber 2011a), and the most recent molecular work hypothesizes a sister relationship of *Physocyclus* and *Artema*, a genus distributed in the Middle East (Dimitrov et al. 2013).

The monophyly of *Physocyclus* has never been tested, but now the taxonomic revision of the genus (Valdez-Mondragón 2010), where 13 new species were described, facilitates an analysis of morphological characters based mainly on the homologies within male chelicerae and female epigyna. The primary objective of the present paper is to test the monophyly of the genus and establish the internal relationships among the species through the first cladistic analysis of the genus *Physocyclus*.

METHODS

Biological material.—The specimens used in this study were the same as those examined by Valdez-Mondragón (2010) and are deposited in the following collections: Colección Nacional de Arácnidos (CNAN), Instituto de Biología, Universidad Nacional Autónoma de México, México; Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia,

Michoacán, México; Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Baja California Sur, México; American Museum of Natural History (AMNH), New York, New York, USA; Texas Memorial Museum (TMM-UT), University of Texas, Austin, Texas, USA; Instituto Nacional de Biodiversidad (INBio), Santo Domingo de Heredia, Costa Rica and Western Australian Museum (WAM), Welshpool, Australia. Other institutions mentioned: California Academy of Sciences (CAS), San Francisco, California, USA; Museum of Comparative Zoology (MCZ), Cambridge, Massachusetts, USA and Muséum National d'Histoire Naturelle, (MNHN) Paris, France. I examined and photographed the specimens with a Nikon SMZ645 stereoscope, following Valdez-Mondragón (2013). The photographs were taken with a Nikon Coolpix S10 VR camera with adapter for the microscope. I used ArcView GIS version 3.2 (Applegate 1999) to prepare distribution maps and edited both the photographs and maps using Adobe Photoshop Version 7.0. Abbreviations used in the figures: E, embolus; ES, embolic sclerites; LAC, lateral apophysis of chelicerae; PP, pore plates; SF, stridulatory files and VAE, ventral apophyses of epigynum.

Taxon sampling.—The cladistic analysis was based on 33 taxa. The ingroup included 29 species of *Physocyclus*. *Physocyclus mexicanus* Banks 1898 was not included in the analysis because this species is known only from the female holotype, which was not examined. *Physocyclus viridis* Mello-Leitão 1940 (insertae sedis) is known only from the male holotype; however, the characters of the original description seem to belong to another genus and not to *Physocyclus* (Valdez-Mondragón 2010), and furthermore the male holotype is lost (B.A. Huber, pers. comm.). The outgroups included *Priscula binghamae* (Chamberlin 1916), *Trichocyclus nigropunctatus* Simon 1908, *Trichocyclus nullarbor* Huber 2001 and *Artema atlanta* Walckenaer 1837. They were selected based on previous phylogenetic relationships with the family Pholcidae (Huber 2000; Bruvo-Madarić et al. 2005; Astrin et al. 2006; Huber 2011a). The trees were rooted on *P. binghamae,* selected because it belongs to the subfamily Modisiminae, which is phylogenetically related to Arteminae (Huber 2011a).

Character matrix.—The character matrix comprised 54 morphological characters, of which 44 were binary and 10 were multistate (Appendix). Forty-seven characters were informative and seven were uninformative, but they were retained in the matrix because they can potentially contribute to future morphological analyses or taxonomic identification keys. Only the important characters used to diagnose the genus *Physocyclus* and the species groups have been illustrated; for all other characters in the genus *Physocyclus* a recent revision should be consulted (Valdez-Mondragón 2010). In the analyses with equal weighting, I deactivated uninformative characters so as not to inflate the tree length and consistency index (CI). The matrix was maintained in WinClada-Asado, version 1.7 (Nixon 2004). I treated multistate characters as non-additive (Fitch 1971).

Cladistic analysis.—The cladistic analysis with equal weighting was run using Heuristic Search under NONA version 1.8 (Goloboff 1993a) and TNT (Goloboff et al. 2008). In NONA, I conducted the analysis with equal weighting using the following commands: Max trees to keep (hold) = 10,000; No. of replications (mult*N) = 1000; Starting trees per replicate (hold/) = 50; using Multiple TBR+TBR (mult*max*). Under TNT, I conducted the analysis with the following commands: Wagner trees: Random seed = 100; Repls. (Number of add. seqs.) = 10,000; Swapping algorithm: Tree Bisection and Reconnection (TBR); trees to save per replication = 100.

I carried out implied character weighting analyses (Goloboff 1993b, 1995) to assess the effects of weighting against homoplastic characters. In TNT, the analysis with implied weighting was conducted using a traditional search with the following commands: Starting trees (Wagner trees): Random seed = 100; No. of replications = 1000; swapping algorithm (TBR); trees to save per replication = 100. Ten arbitrary values for the concavity constant were used: K = 1–10.

Branch support was calculated using Jaekknife (Farris et al. 1996) under TNT (Goloboff et al. 2008) and the command: Number of replicates = 1000, removal probability = 36%, using traditional search, and Bremer support (Bremer 1988) under TNT, retain trees suboptimal by 5 steps.

I resolved ambiguous character optimizations with accelerated transformation (ACCTRAN) (Farris 1970; Swofford & Maddison 1987; Agnarsson & Miller 2008). The trees were edited in WinClada and Adobe Photoshop 7.0.

RESULTS

Heuristic equal weighting searches in NONA and TNT found 12 most parsimonious cladograms (L = 127, CI = 70, RI = 85). Figure 1 shows the strict consensus of the minimum length trees in which six nodes collapsed. The cladistic analysis supports the monophyly of the genus *Physocyclus* Simon 1893 with high Jackknife and Bremer values (Fig. 1), and the genus is supported by three synaphomorphies (char. 7, 8, 12) (see discussion for character states). The analysis found two clades within the genus *Physocyclus*, considered as species groups and supported with high Jackknife and Bremer values (Fig. 1). The *globosus* species group consists of 11 species, and the *dugesi* group consists of 19 species (Fig. 1).

The monophyly of the *globosus* group is supported with high Jackknife and Bremer values of 74% and 4 respectively, and by five synapomorphies (Fig. 1): 1) the posterior dorsal sclerotized protuberance on carapace of the female (char. 3) (right arrow, Fig. 14); 2) the sclerotized patch on dorsal anterior part on the female opisthosoma (char. 4) (left arrow, Fig. 14); 3) the short, wide, and oval-shaped pore plates in the epigynum (char. 10, character state 2) (Figs. 17, 21); 4) the dorso-distal spine on the embolus (char. 30) (arrow, Fig. 19) and 5) the embolic sclerites positioned dorsally on the embolus (char. 37) (left arrow, Fig. 15). The monophyly of the *dugesi* group is supported with high Jackknife and Bremer support values of 82% and 5 respectively, and by four synapomorphies (Fig. 1): 1) the lateral constraints in middle part of the epigynum are very marked and bell-shaped (char. 9, character state 1) (arrow, Fig. 2), 2) the embolic sclerites on retrolateral part of the bulb (char. 43) (left arrow, Fig. 8), 3) the notch between embolic sclerites and embolus (char. 45) (middle arrow, Fig. 6) and 4) by having > 30 sclerotized cones frontally on male chelicerae (except *P. platnicki*: Valdez-Mondragón 2010, Fig. 197) (char. 23, character state 1) (Figs. 5, 9).

The analyses with implied weighting, using ten different concavity values (K), also supported the monophyly of the

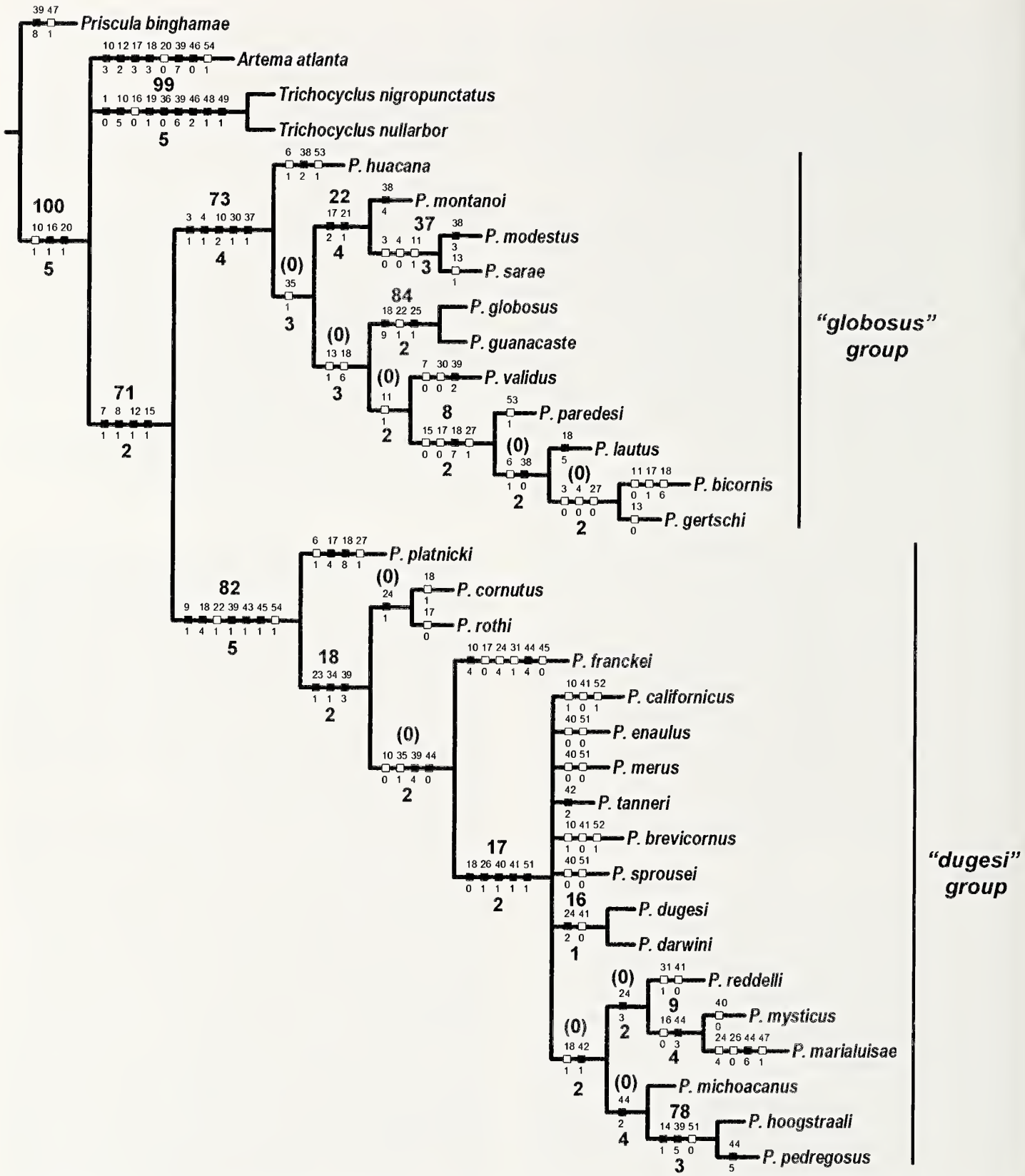
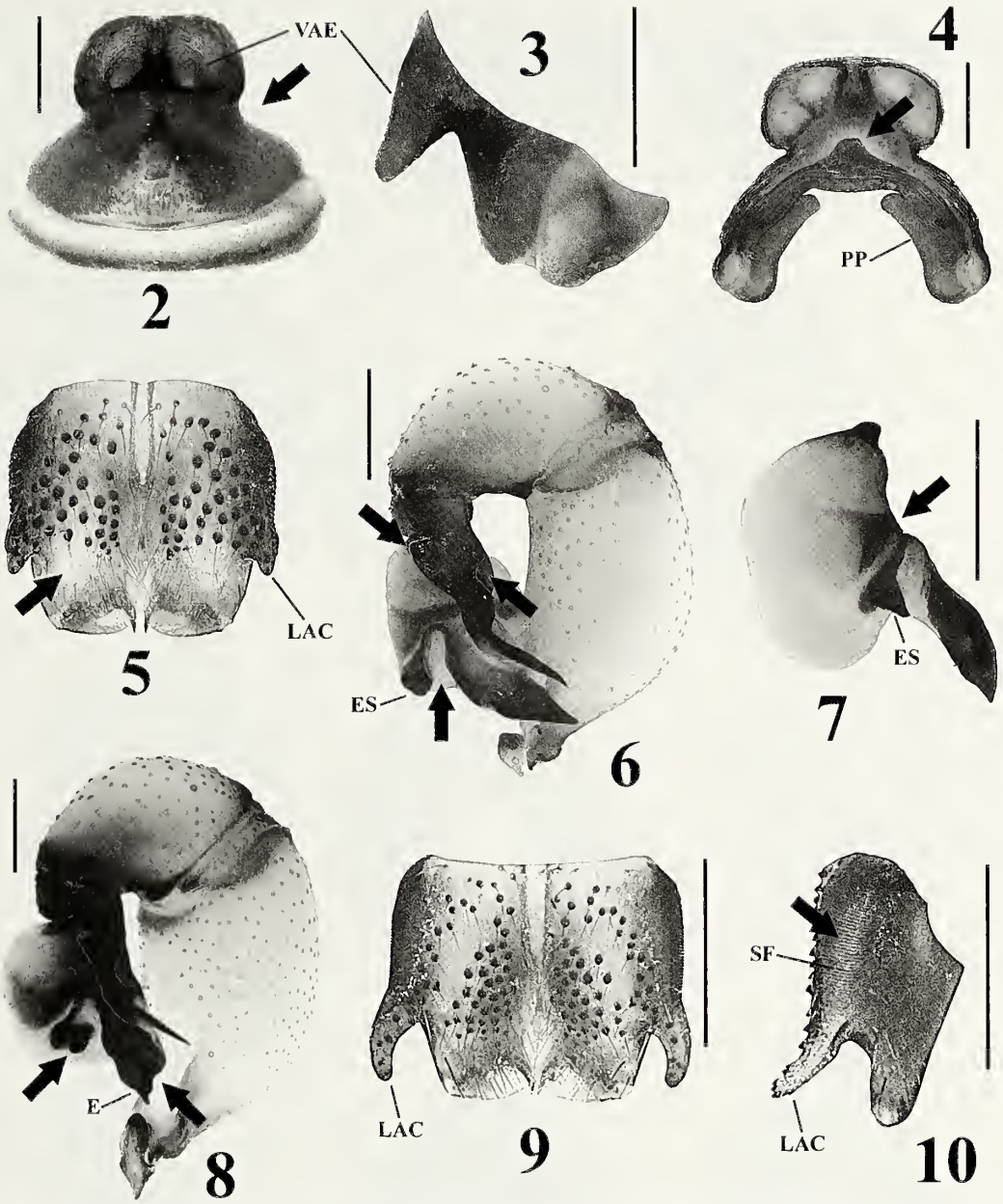


Figure 1.—Strict consensus trees of 12 most parsimonious trees obtained by cladistic analysis with equal weighting of characters under NONA (L= 127, CI=70, RI= 85). Black bars indicate unreversed synapomorphic or apomorphic states, and white bars indicate homoplastic characters. Small numbers above bars indicate character number; small numbers below bars indicate character state. Larger numbers above branches indicate Jackknife support values; larger numbers below branches indicate Bremer support values; (0) above nodes indicate unsupported or collapsed nodes with Jackknife.



Figures 2–10.—*Physosyclus cornutus*: **2**, **3**. Epigynum, ventral and lateral view respectively (arrow indicates lateral constraints in middle part). *P. michoacanus*: **4**. Epigynum, dorsal view (arrow indicates the single sclerotized projection on the arc). *P. dugesi*: **5**. Chelicerae, frontal view (arrow indicates the pale concavity on each chelicerae); **6**. Left palp, retrolateral view (left arrow indicates dorsal apophysis of procursus; middle arrow indicates the notch between embolic sclerites and embolus; right arrow indicates the ventral notch of the procursus); **7**. Bulb of the left palp, dorsal view (arrow indicates the sclerotized retrolateral region strongly visible). *P. sporusei*: **8**. Left palp, retrolateral view (left arrow indicates the embolic sclerites, right arrow indicates the apical ventral concavity). *P. reddelli*: **9**, **10**. Chelicerae, frontal and lateral views, respectively (arrow indicates the stridulatory file). Scale bars: 0.5 mm.

genus *Physocyclus*, with Jackknife values of ≥ 70 (Table 1). The analyses with concavity values (K = 6–10) obtained fewer parsimonious trees (5 or 6) of the same length as the analysis of equal weighting (Table 1). The analyses with the highest fit values (K = 9, 10) recovered only five parsimonious trees, with the same CI and RI values of the equally weighted trees (Table 1), and the strict eonsensus found the same topology as with equal weighting (Fig. 1).

Morphological characters.—The morphological characters used in the phylogenetic analysis [54 characters (44 binary and 10 multistate)] are listed below; some characters are described in Valdez-Mondragón (2010), which is abbreviated in this part as VM (2010):

Prosoma:

1. Anterior median eyes, diameter: (0) > diameter of anterior lateral eyes, (1) < diameter of anterior lateral eyes
2. Fovea, shape: (0) point-shaped, (1) longitudinal
3. Carapace of female, posterior dorsal sclerotized protuberance (arrow, Fig. 14): (0) absent, (1) present

Table 1.—Summary of the phylogenetic hypotheses among the most parsimonious trees (MPT) found with equal weighting (EW) and implied weighting (IW), with 10 values for the concavity constant (*K*), arranged in order of increasing fit (*fi*). CI= Consistency index, RI= Retention index, J= Jackknife values that support the monophyly of *Physocyclus* in the different hypothesis.

Analyses	MPT	Steps	fit (*fi*)	CI	RI	Status of *Physocyclus*
IW: *K*= 1	42	133	33.52	67	82	J= 70 (monophyletic)
IW: *K*= 2	14	128	36.97	70	84	J= 71 (monophyletic)
EW	12	127	38.90	70	85	J= 72 (monophyletic)
IW: *K*= 3	14	128	39.10	70	84	J= 72 (monophyletic)
IW: *K*= 4	14	128	40.48	70	84	J= 73 (monophyletic)
IW: *K*= 5	14	128	41.44	70	84	J= 74 (monophyletic)
IW: *K*= 6	5	127	42.17	70	85	J= 74 (monophyletic)
IW: *K*= 7	6	127	42.73	70	85	J= 75 (monophyletic)
IW: *K*= 8	6	127	43.17	70	85	J= 75 (monophyletic)
IW: *K*= 9	5	127	43.53	70	85	J= 75 (monophyletic)
IW: *K*= 10	5	127	43.83	70	85	J= 76 (monophyletic)

Opisthosoma:

4. Opisthosoma of female, sclerotized patch, on dorsal anterior part (arrow, Fig. 14): (0) absent, (1) present
5. Epigynum, distal paired apophysis, next to epigastric furrow: (0) absent, (1) present
6. Epigynum, two small median U-shaped concavities (VM 2010; Figs. 67, 144): (0) absent, (1) present
7. Epigynum, paired ventral apophysis on anterior part (Figs. 2, 3, 11, 20): (0) absent, (1) present
8. Epigynum, lateral constraints in middle part (Figs. 2, 20): (0) absent, (1) present
9. Epigynum, lateral constraints in middle part, shape: (0) barely visible, inconspicuous (Fig. 20); (1) very marked, bell-shaped (arrow, Fig. 2)
10. Epigynum, pore plates, shape: (0) very long and thin (Fig. 4); (1) long and wide (VM 2010; Figs. 13, 20); (2) short, wide, oval-shaped (Fig. 17); (3) short and thin; (4) triangular; (5) short and curved
11. Pore plates, structures bag-shaped below them (arrow, Fig. 21): (0) absent, (1) present
12. Epigynum, sclerotized arc, dorsal view: (0) without sclerotized projection on anterior part, (1) with a sclerotized projection on anterior part (arrows, Figs. 4, 17), (2) with two sclerotized projections on anterior part
13. Epigynum, dorsal arc surrounding pore plates (Fig. 17): (0) absent, (1) present
14. Epigynum, median protuberances, laterally (VM 2010; Figs. 60, 95): (0) absent, (1) present

Legs:

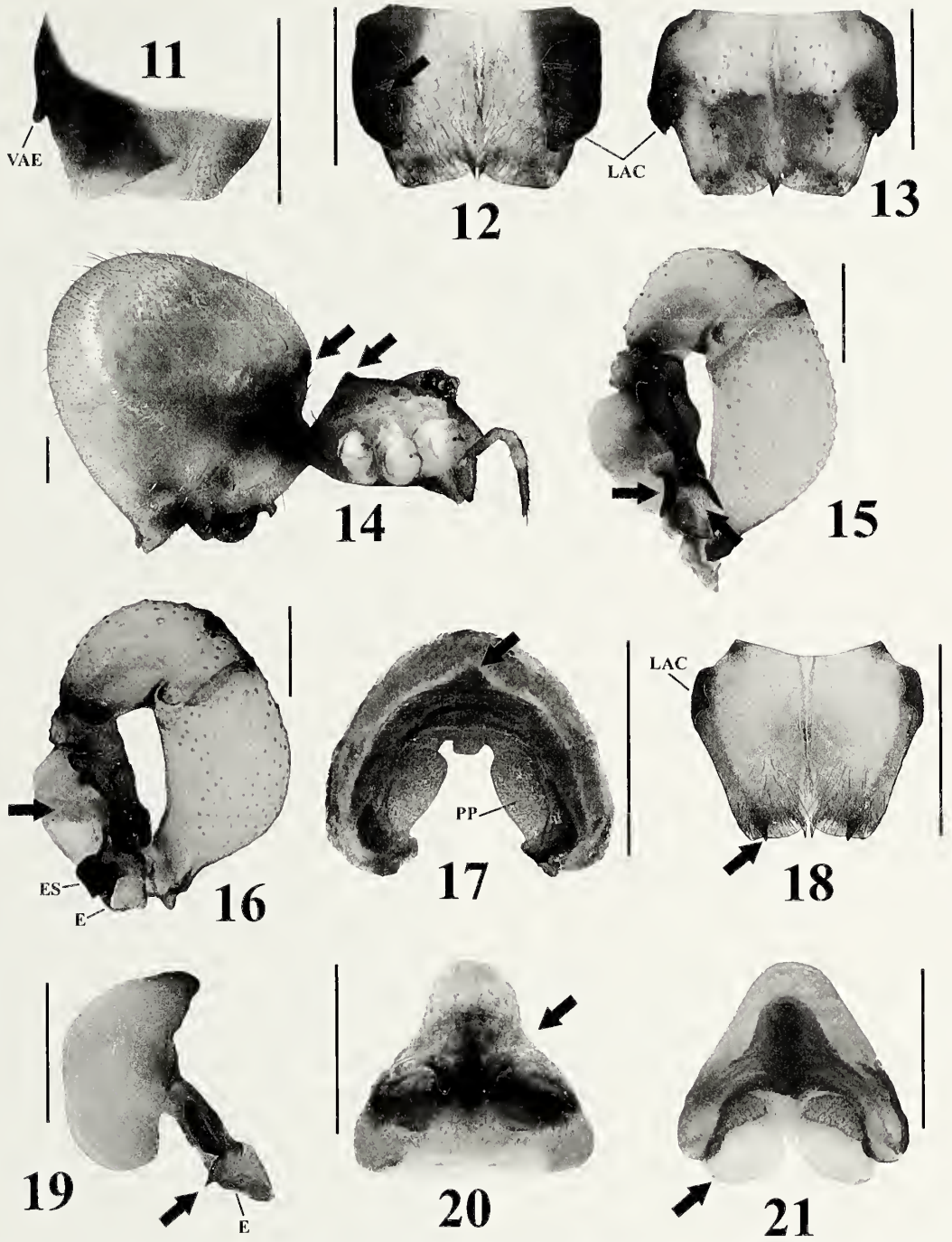
15. Legs, curved setae in tibiae and metatarsi: (0) absent; (1) present

Chelicerae:

16. Chelicerae of male, lateral apophysis (Figs. 5, 9, 12, 13): (0) absent, (1) present
17. Chelicerae of male, lateral apophysis, location: (0) proximal (Fig. 18), (1) middle part (Fig. 5), (2) frontal-retrolateral (arrow, Fig. 12), (3) along, (4) distally (VM 2010; Fig. 197)
18. Chelicerae of male, lateral apophysis, shape: (0) small and conical (Fig. 5), (1) conical and long (Figs. 9, 10), (2) shield-shaped (Fig. 12), (3) wide and along, (4) wide and projected toward front (VM 2010; Fig. 183), (5) small and triangular (Fig. 18), (6) wide and triangular in lateral view (VM 2010; Figs. 2, 113), (7) wide and with two projections in lateral view (VM 2010; Fig. 141), (8) small, with several cones distally (VM 2010; Figs. 197, 198), (9) small and with irregular shape (Fig. 13)
19. Chelicerae of male, frontal curved apophysis, basally: (0) absent, (1) present
20. Chelicerae of male, stridulatory files laterally (arrow, Fig. 10): (0) absent, (1) present
21. Chelicerae of male, discontinuous files frontally: (0) absent, (1) present, on wide apophysis shield-shaped (arrow, Fig. 12)
22. Chelicerae of male, sclerotized cones frontally (Figs. 5, 9): (0) absent, (1) present
23. Chelicerae of male, sclerotized cones frontally, number on each chelicerae: (0) < 20 cones (Fig. 13), (1) > 30 cones (Figs. 5, 9)
24. Chelicerae of male, > 30 sclerotized cones frontally, position: (0) on basal half, and on prolateral part of chelicerae and lateral apophysis (VM 2010, Fig. 8); (1) on basal half, and on prolateral part of chelicerae and lateral apophysis, leaving a basal zone on prolateral part without cones (VM 2010, Fig. 15); (2) on ¾ of total length, and on prolateral part of chelicerae and lateral apophysis (Fig. 5); (3) on prolateral part, and toward prolateral part of lateral apophysis leaving an area with half-moon shape without cones between them (Fig. 9); (4) scattered throughout (VM 2010; Fig. 119)
25. Chelicerae of male in frontal part, pale basal half and brown distal half (Fig. 13): (0) absent, (1) present
26. Chelicerae of male, pale concavity on each chelicera (arrow, Fig. 5): (0) absent, (1) present
27. Chelicerae of male, frontal distal small apophysis, conical (arrow, Fig. 18): (0) absent, (1) present
28. Chelicerae of male, retrolateral frontal apophysis, near to the fangs (*Priscula binghamae*): (0) absent, (1) present

Palps:

29. Procursus, dorsal apophysis and ventral notch basally (left and right arrows respectively, Fig. 6): (0) absent, (1) present



Figures 11–21.—*Physocyclus modestus*: **11**. Epigynum, lateral view; **12**. Chelicerae, frontal view (arrow indicates the frontal-retrolateral apophysis on chelicerae). *P. guanacaste*: **13**. Chelicerae, frontal view. *P. globosus*: **14**. Female habitus, lateral view (left arrow indicates the sclerotized patch on dorsal part of opisthosoma, right arrow indicates the posterior dorsal sclerotized protuberance on carapace); **15**. Left palp, retrolateral view (left arrow indicates the embolic sclerites, right arrow indicates the brush of pseudotrichia on procursus). *P. bicornis*: **16**. Left palp, retrolateral view (left arrow indicates the inconspicuous sclerotized retrolateral region on palp bulb); **17**. Epigynum, dorsal view (arrow indicates the single sclerotized projection on the arc). *P. lautus*: **18**. Chelicerae, frontal view (arrow indicates frontal distal small apophysis); **19**. Bulb of the left palp, dorsal view (arrow indicates the dorso-distal spine on embolus). *P. sarae*: **20**. Epigynum, ventral view (arrow indicates lateral constraints in middle part); **21**. Epigynum, dorsal view (arrow indicates the bag-shaped structures below the pore plates). Scale bars: 0.5 mm.

30. Embolus, dorso-distal spine (arrow, Fig. 19): (0) absent, (1) present
31. Femora of male palp, small prolateral ventral apophysis, with cone-shaped (VM 2010; Fig. 121): (0) absent, (1) present
32. Femora of male palp, prolateral ventral apophysis, distally, oval with flat tip: (0) absent, (1) present
33. Femora of male palp, large ventral conical projection (VM 2010; Fig. 86): (0) absent, (1) present

34. Bulb, sclerotized retrolateral region: (0) absent or inconspicuous (arrow, Fig. 16), (1) strongly visible (arrow, Fig. 7)
35. Procursus, large distal spine (Figs. 6, 8, 15): (0) absent, (1) present
36. Procursus, brush of pseudotrichia distally (right arrow, Fig. 15): (0) absent, (1) present
37. Embolus, embolic sclerites dorsally (left arrow, Fig. 15): (0) absent, (1) present
38. Embolus, embolic sclerites dorsally, shape: (0) large and wide, on almost total length of embolus (VM 2010; Fig. 65); (1) small, on almost total length of embolus, without notch on median part (Fig. 15); (2) long and oval distally, located on basal part of embolus (VM 2010; Fig. 149); (3) small, with notch on median part (VM 2010; Fig. 79); (4) small, projected further than total length of embolus (VM 2010; Fig. 156)
39. Embolus, shape: (0) almost square-shaped distally (Fig. 16); (1) long, with "J"-shape (VM 2010, Fig. 199); (2) long, with upside down "S"-shape (VM 2010; Fig. 114); (3) rounded apically (VM 2010; Fig. 17); (4) triangular-shaped apically (Fig. 6); (5) triangular-shaped dorsally and rounded-shaped ventrally (VM 2010; Fig. 58); (6) conical and oval distally; (7) curved distally; (8) sigmoidal
40. Embolus, with triangular-shaped apically, position: (0) pointing in diagonal position to the longitudinal axis of femur (Fig. 8), (1) pointing in perpendicular position to the longitudinal axis of femur (Fig. 6)
41. Embolus, triangular-shaped apically, with apical ventral concavity (right arrow, Fig. 8): (0) absent, (1) present
42. Embolus, apical ventral concavity, shape: (0) small (right arrow, Fig. 8), (1) curved and long (VM 2010; Fig. 86), (2) circular and large (VM 2010; Fig. 107)
43. Bulb, embolic sclerites on retrolateral part (left arrow, Fig. 8): (0) absent, (1) present
44. Bulb, embolic sclerites on retrolateral part, shape: (0) small and triangular (left arrow, Fig. 8); (1) long and wide (VM 2010; Fig. 17); (2) long and thin (VM 2010; Fig. 58); (3) small and oval (VM 2010; Fig. 86); (4) long and triangular (VM 2010; Fig. 121); (5) small, wide and curved (VM 2010; Fig. 93); (6) long and curved (VM 2010; Fig. 164)
45. Bulb, notch between embolic sclerites and embolus (middle arrow Fig. 6): (0) absent, (1) present
46. Procursus, shape: (0) square (wider than long), (1) conical (wider basally than distally) (Figs. 6, 8, 15, 16), (2) curved (*Trichocyclus*)
47. Male palp, ventral apophysis distally on femur (VM 2010; Fig. 163): (0) absent, (1) present
48. Procursus, long rounded protuberance, ventrally (*Trichocyclus*): (0) absent, (1) present
49. Procursus, dorsal deep concavity (*Trichocyclus*): (0) absent, (1) present
50. Procursus, dorsal projection in middle part (*Priscula*): (0) absent, (1) present
51. Embolus, dorsal projection: (0) absent, (1) present
52. Embolus, dorsal projection, shape: (0) present, curved; (1) present, circular, clearly visible (VM 2010; Fig. 135)
53. Embolus, distal spine (VM 2010; Figs. 149, 206): (0) absent, (1) present
54. Embolus, retrolateral part: (0) white, poorly chitinized (Figs. 15, 16); (1) black, strongly chitinized (Figs. 6, 8)

TAXONOMY

Pholcidae C.L. Koch 1850
Physocyclus Simon 1893

Physocyclus Simon 1893:1(2), 257–488.

Type species.—*Pholcus globosus* Taczanowski 1874:105 (description ♀).

Diagnosis.—Distinguished from other pholcid genera by the combination of the following characters: epigynum with paired ventral apophysis on anterior part (Figs. 2, 3, 11, 20), epigynum with lateral constraints in middle part (arrows, Figs. 2, 20), epigynum with internal sclerotized arc with a sclerotized projection on anterior part (arrows, Figs. 4, 17), male chelicerae with lateral apophysis (Figs. 5, 9, 10, 12, 13), male palp with enlarged femur (Figs. 6, 8, 15, 16), male chelicerae with sclerotized cones frontally (> 30 cones in the *dugesi* group) (Figs. 5, 9). However, cones in the *globosus* group only present on *P. globosus* and *P. guanacaste* (0–20 cones) (Fig. 13).

Description.—Medium-sized spiders (total length 3–7 mm). Carapace usually light yellow, light brown, or with orange undertones, most species with marginal dorsal spots. Fovea with irregular pattern around it, gray or brown. Fovea forming a "Y" with posterior part of ocular region. Eight eyes on ocular region slightly high. Clypeus broad, gently sloping, in some species with two brown, gray or orange lines. Male chelicerae with lateral apophysis (except *P. mysticus* and *P. marialuisae*), apophysis variable in shape and size (Figs. 5, 9, 10, 12, 13, 18). Female chelicerae simple, without apophysis. Male chelicerae with lateral stridulatory files (Fig. 7); females of some species have lateral stridulatory files, but always smaller than the male. Male chelicerae with sclerotized cones on most of the species, variable in number and position (Figs. 5, 9). Male palp femur wide (Figs. 6, 8, 15, 16). Procursus long, dark, sclerotized, with brush of pseudotrichia (right arrow, Fig 15) and spine distally (Figs. 6, 8). Embolic sclerites with different shape and position (arrows, Figs. 8, 15; Valdez-Mondragón 2010, Figs. 10, 51, 72, 93). Embolus in retrolateral part of bulb, sclerotized, with variable shape (Figs. 6–8, 15, 16, 19), sperm duct opening distal-dorsally. Female palp simple. Sternum and labium wider than long, some species have sternum with gray, brown or dark orange spots. Endites long. Males with legs longer than females, femora with rings sub-distally, tibiae with basal and sub-distal rings, more visible in some species than others. Color on legs variable, pale or dark yellow, pale or dark orange, basal part of femora paler than the other segments, metatarsi and tarsi darker than the other segments. Legs without spines. Male legs with curved setae on tibiae and metatarsi. Opisthosoma globular (Fig. 14), thicker than long, larger in females than in males, with lateral and dorsal irregular spots, brown, white or gray. Epigynum with paired anterior ventral apophysis, with variable size and shape (Figs. 2, 3, 11, 20; Valdez-Mondragón 2010, Figs. 5, 33–38). Epigynum wider than long in most species, bell-shaped (Figs. 2, 20; Valdez-Mondragón 2010, Figs. 26, 67, 137). Epigynum with pore plates variable in

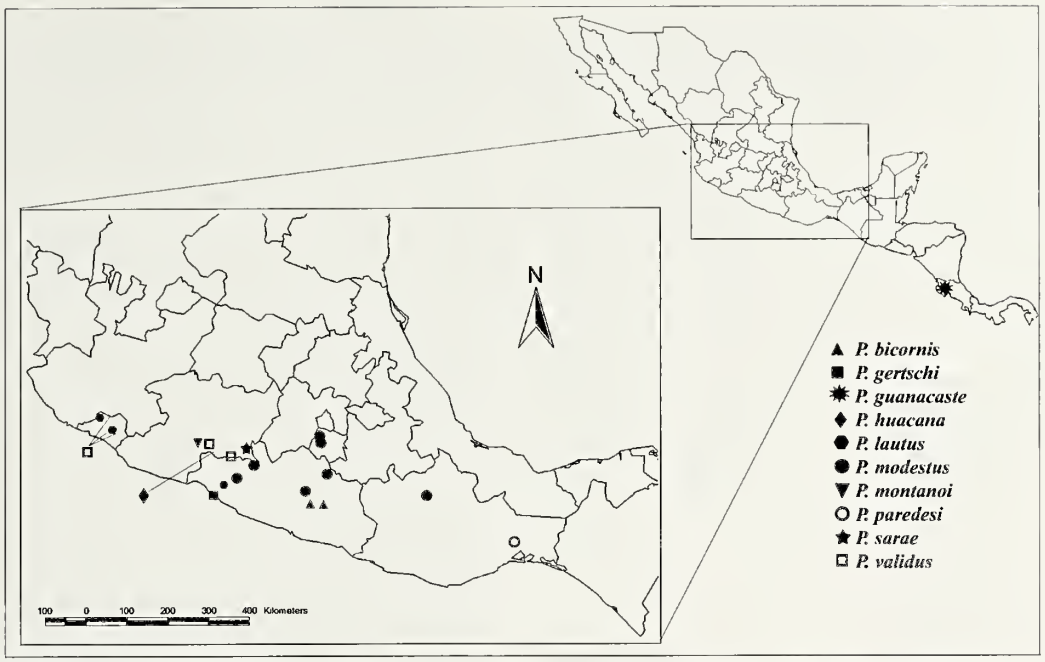


Figure 22.—Natural distribution of the species of the *globosus* group. *Physocyclus globosus* is not plotted because it is introduced in several places around the world.

size, position and shape depending on the species (Figs. 4, 17, 21; Valdez-Mondragón 2010, Figs. 27, 117, 159).

Monophyly.—The genus *Physocyclus* is defined by the following synapomorphies: 1) epigynum with paired ventral apophysis on anterior part (Figs. 2, 3, 11, 20), 2) epigynum with lateral constraints in middle part (arrows, Figs. 2, 20), and 3) epigynum with internal sclerotized arc with a sclerotized projection on anterior part (arrows; Figs. 4, 17).

Composition.—The genus *Physocyclus* is composed of 30 species in two species groups (*globosus* and *dugesi*). The *globosus* group (11 species): *P. globosus* (Taczanowski 1874), *P. bicornis* Gertsch 1971, *P. lautus* Gertsch 1971, *P. modestus* Gertsch 1971, *P. validus* Gertsch 1971, *P. guanacaste* Huber 1988, *P. gertschi* Valdez-Mondragón 2010, *P. huacana* Valdez-Mondragón 2010, *P. montanoi* Valdez-Mondragón 2010, *P. paredesi* Valdez-Mondragón 2010 and *P. sarae* Valdez-Mondragón 2010. The *dugesi* group (19 species): *P. dugesi* Simon 1893, *P. mexicanus* Banks 1898, *P. cornutus* Banks 1898, *P. tanneri* Chamberlin 1921, *P. mysticus* Chamberlin 1924, *P. enaulus* Crosby 1926, *P. californicus* Chamberlin & Gertsch 1929, *P. hoogstraali* Gertsch & Davis 1942, *P. merus* Gertseh 1971, *P. pedregosus* Gertsch 1971, *P. reddelli* Gertsch 1971, *P. brevicornus* Valdez-Mondragón 2010, *P. darwini* Valdez-Mondragón 2010, *P. franckei* Valdez-Mondragón 2010, *P. marialuisae* Valdez-Mondragón 2010, *P. michoacanus* Valdez-Mondragón 2010, *P. platnicki* Valdez-Mondragón 2010, *P. rothi* Valdez-Mondragón 2010 and *P. sprousei* Valdez-Mondragón 2010. Although *P. mexicanus* was not part of the analysis, it was included in *dugesi* group because the female holotype has a long ventral apophysis on the female epigynum as do the other species of the group.

Natural history.—Species such as *P. globosus, P. dugesi* and *P. enaulus* have been collected in houses and buildings (Rodríguez-Márquez & Peretti 2010, Valdez-Mondragón 2010); human activity is responsible for the wide geographic distribution of these species. Synanthropic species occupy corners of ceilings of rooms, basements, bathrooms, under sinks, under tables and benches, under stored items and furniture, and under drains for drainage of roads, in dark warm places without wind currents and with little disturbance. Some species (*P. franckei, P. dugesi, P. enaulus, P. hoogstraali, P. merus, P. pedregosus, P. tanneri*, and *P. reddelli*) inhabit dry semiarid climates, while others (*P. huacana, P. modestus, P. validus, P. paredesi, P. bicornis, P. californicus, P. cornutus, P. michoacanus, P. brevicornus*, and *P. dugesi*) prefer tropical deciduous forest, between 0–1900 m elevation. Above 1900 m elevation, they have been collected only in buildings. Scientists have never collected the genus in temperate climatic zones such as pine, oak or pine-oak forest, which are the natural habitat for other genera such as *Ixchela* Huber 2000 (Valdez-Mondragón 2013). In karst zones, it is common to find them because of their troglophilic habits; some species have been collected in the entrances of caves and inside on crevices in walls and on formations (stalactites, stalagmites and columns). This is the case for *P. bicornis, P. dugesi, P. enaulus, P. franckei, P. hoogstraali, P. lautus, P. merus, P. modestus, P. pedregosus, P. reddelli, P. tanneri*, and *P. validus*. Outside the caves, their natural habitat is in rock walls, between rocks and dark crevices, with high humidity, warm temperature, and protection from strong wind drafts. Bridges and culverts under roads and railroad tracks are excellent collecting spots.

Distribution.—*Physocyclus* has a natural distribution in North America, with most of the species known found in Mexico (Figs. 22, 23), with *P. californicus, P. enaulus, P. hoogstraali,* and *P. tanneri* distributed in the southern part of the United States, and *P. guanacaste* distributed in Costa Riea. *P. dugesi* has been introduced into Costa Rica and Venezuela, although this last record of Caporiacco (1955) could be

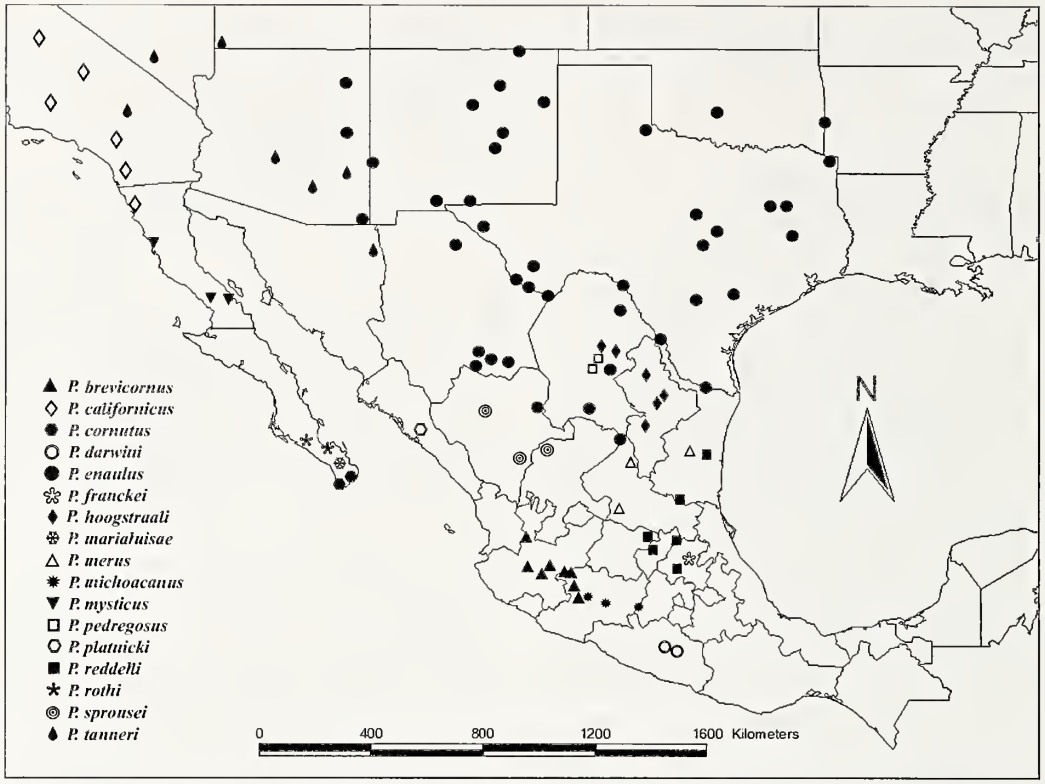


Figure 23.—Natural distribution of the species of the *dugesi* group. *Physocyclus dugesi* is not present because it is an introduced species in Central and South America.

erroneous (B. Huber pers. comm.). *P. globosus* has been introduced and reported in different countries around the world (Valdez-Mondragón 2010).

DISCUSSION

The subfamily Arteminae proposed by Huber (2011) is composed of the genera *Holocneminus*, *Artema, Tibetia, Physocyclus* and *Trichocyclus*; and previous studies have always supported the subfamily (Huber 2001; Bruvo-Madarić et al. 2005). The paired dorsal apophysis and the ventral notch basally on the procursus of the male palp (char. 29) (Huber 2000, 2001) define this subfamily. Recently, Dimitrov et al. (2013), using molecular data, also transferred the genera *Nita* and *Wugigarra* (previously in Modisiminae) to Arteminae. *Wugigarra* also has a paired dorsal apophysis and a basal ventral notch on the procursus of the male palp (B. Huber pers. comm.; A. Valdez-Mondragón pers. obs.), as the do rest of the genera of the subfamily.

In this analysis, I found a trichotomy among *Artema*, *Trichocyclus*, and *Physocyclus* (Fig. 1); Dimitrov et al. (2013) considered *Physocyclus* and *Trichocyclus* to be sister taxa based on the reduction of the epiandrous spigots and preliminary molecular evidence (Huber 2001; Bruvo-Madarić et al. 2005).

The monophyly of the genus *Physocyclus* is supported by three synapomorphies, with high Jackknife and Bremer values that support the genus at 72% and 2 respectively (Fig. 1). The first synapomorphy is the paired ventral apophysis on the anterior part of the epigynum (char. 7) (Figs. 2, 3, 11, 20). However, the apophyses of the species differ in shape, and each species has a particular diagnostic shape. There were two apophysis patterns: conical and short in the *globosus* group (Figs. 11, 20) and long, curved and wide in most of the species in the *dugesi* group (Figs. 2, 3), but these apophyses are absent on *P. validus*, likely a secondary loss (Valdez-Mondragón 2010, Fig. 116). The second character is the lateral constraints in the middle part of the epigynum (char. 8) (arrows, Figs. 2, 20). This character is inconspicuous or barely visible on the species of the *globosus* group (char. 9, character state 0) (Fig. 20), whereas in the *dugesi* group, the shape is very marked (char. 9, character state 1) (Fig. 2). The third character is the arc of the uterus with a single sclerotized projection on its anterior part (char. 12, character state 1) (arrows, Figs. 4, 17), although it is hard to tell if this simple sclerotized projection belongs only to *Physocyclus* or may be present in other genera of Arteminae. At least in *Artema*, this character is present but with two sclerotized projections (char. 12, character state 2), whereas in *Priscula*, *Trichocyclus*, and *Wugigarra* (Huber 2001, Figs. 9, 27, 42), this sclerotized projection is absent. Although the analysis found that a fourth character supporting the monophyly of *Physocyclus* is the curved setae on the tibiae and metatarsi of the legs (char. 15), this character has evolved several times convergently within the subfamilies Arteminae, Modisiminae and Smeringopinae (Huber 2000, 2011). This character was unknown in *P. guanacaste, P. lautus,* and *P. gertschi* due to bad specimen preservation and missing legs, and even these curved setae are absent in *P. paredesi* and *P. bicornis.*

Globosus group: The phylogenetic relationships among the species of this group were the same in the 12 most parsimonious trees found in the analysis (Fig. 1). The monophyly of the group is supported by five synapomorphies.

Characters 3 and 4 are a functional unit because these structures are in contaet when the female moves its opisthosoma toward the prosoma. However, these characters were coded as different because they were treated as just one character. The analysis found 24 most parsimonious trees, collapsing nine clades with lower values of Ci and Ri. Although the shape of the pore plates is variable in the different species of both groups (char. 10), all species of the *globosus* group share the oval shape (Figs. 17, 21). The dorso-distal spine on the embolus (char. 30) is absent on *P. validus* and can be considered a reversal. In the other species, this spine has different sizes and shapes, whieh make eodification difficult. The dorsal embolic sclerites on the embolus (char. 37) (left arrow, Fig. 15) in the *globosus* group are present on all species. However, the shape of the embolic sclerites (char. 38), which was coded as a multistate character, varies. Only the character state (0): large and wide, almost the total length of the embolus, is shared [*P. lautus* (*P. bicornis* + *P. gertschi*)] (Figs. 1, 16). The large distal spine on the procursus (char. 35) (Figs. 6, 8) is present in all species of the group except *P. huacana*. This character apparently appeared convergently twice, in the *globosus* group (except *P. huacana*) and in most of the species of the *dugesi* group, except *P. platnicki, P. cornutus* and *P. rothi* (Fig. 1).

The clade [*P. montanoi* (*P. modestus* + *P. sarae*)] is supported by the lateral apophysis of male chelicerae in a frontal-retrolateral position (char. 17, character state 2) and male chelicerae with discontinuous files on the wide, shield-shaped apophysis (char. 21, character state 1) (arrow, Fig. 12). This clade had a low Jackknife support value (22%), but a high Bremer support value of 4 (Fig. 1). The position of the lateral apophysis of the male chelicerae was coded as multistate because in both groups the position varies: proximal (character state 0), on the middle part (1), lateral (3) or distally (4). Although the diseontinuous files on the apophysis of the male chelicerae support the clade [*P. montanoi* (*P. modestus* + *P. sarae*)], the lateral stridulatory files (char. 20) apparently have evolved convergently several times in different genera of subfamilies Ninetinae, Arteminae and Smeringopinae, except in Modisiminae and Pholcinae (Huber 1995, 2000, 2011a).

Physocyclus globosus + *P. guanacaste* are sister species. Although the shape of the lateral apophysis of the male chelicerae (char. 18) is a multistate character with nine charaeter states, in both species it is small and irregular (character state 9) (Fig. 13). Besides, *P. globosus* + *P. guanacaste* was the only close relationship in the group with high Jackknife and Bremer support values of 84% and 2, respectively (Fig. 1). Another synapomorphy that supports *P. globosus* + *P. guanacaste* is the male chelicerae with a pale basal half and a brown distal half (char. 25) (Fig. 13).

Coding of the apophysis shape of the male chelicerae was difficult due to the variation in the two groups; some of the character states were even autapomorphies for certain species, such as *P. platnicki* and *P. lautus*. This character is also absent in *P. mysticus* and *P. marialuisae* and is considered a reversal. Similarly challenging was the coding of the position of the lateral apophysis of the male chelicerae (char. 17), it being a multistate and homoplastic character (Fig. 1). The bag-shaped structures below each pore plate (char. 11) (arrow, Fig. 21) were found in some species of this group. These structures have apparently evolved convergently twice in the clade *P. modestus* + *P. sarae*, and in the clade composed from *P. validus* to *P. gertschi* (Fig. 1). This character is a reversion in *P. bicornis*.

Although the dorsal embolic sclerites consisted of several shapes (char. 38), they were large and wide for almost the total length of the embolus (character state 0) (Fig. 16), supporting the clade [*P. lautus* (*P. bicornis* + *P. gertschi*)]. In some cases, the character states are diagnostie for such species as *P. modestus*, *P. huacana* and *P. montanoi* (Valdez-Mondragón 2010: Figs. 79, 150, 156). Finally, the conical frontal-distal apophysis on the male ehelicerae (char. 27) (arrow, Fig. 18; Valdez-Mondragón 2010, Figs. 197, 204) is a character that may have appeared convergently twice in both species groups because it is present in *P. paredesi* and *P. lautus* (*globosus* group) and *P. platnicki* (*dugesi* group).

Dugesi group: In comparison with the *globosus* group, there were changes in the relationships among the species of the *dugesi* group in the 12 most parsimonious trees, with the strict consensus showing the internal relationships in the group (Fig. 1). The monophyly of the group is supported by four synapomorphies. Although the shape of the lateral male chelicerae (char. 18) (Fig. 5, 9, 10) seems to support the group, this character has several character states, one being that the lateral apophysis is small and conical (character state 0) (Fig. 5), the shape that is shared among most of the species (*P. californicus, P. enaulus, P. merus, P. brevicornus, P. sprousei, P. dugesi, P. darwini* and *P. tameri*). The small, conical lateral apophysis is a plesiomorphic or ancestral state, whereas conical and long (character state 1) (Figs. 9, 10) is a derived state, shared with *P. reddelli, P. michoacanus, P. hoogstraali,* and *P. pedregosus*, but absent on *P. mysticus* and *P. marialuisae* (Valdez-Mondragón 2010; Figs. 84, 161). The embolus shape (char. 39) seems to support the group. However, four different character states were coded, one being an apically triangular embolus (character state 4) (Fig. 6), the state shared in the most of the species except *P. platnicki, P. cornutus, P. rothi, P. hoogstraali*, and *P. pedregosus*. Another character that supports the group is the strongly visible, sclerotized retrolateral region around the male bulb (char. 34, character state 1) (arrow, Fig. 7), absent in *P. platnicki*. About the shape of embolic sclerites on the retrolateral part of bulb (char. 44), apparently the plesiomorphic state was characterized by the species that share long and wide embolic sclerites (character state 1) (Valdez-Mondragón; Figs. 17, 185), whereas the derived state in most of the species was defined by small, triangular embolic sclerites (character state 0) (Figs. 6, 7; left arrow Fig. 8).

Four synapomorphies support the largest clade within the group from *P. californicus* to *P. pedregosus* (Fig. 1); however, this clade is weakly supported with a low Jackknife value of 17%, although supported by with Bremer values of 2. The first synapomorphy is the pale concavity on each chelicera of the male (char. 26) (arrow, Fig. 5) (absent on *P. marialuisae*). The second synapomorphy is the apical position of the triangular-shaped embolus (char. 40); although this is a multistate character, most of the species have a triangular embolus pointing in a perpendicular position to the longitudinal axis of the femur (character state 1) (Fig. 6). Although there is a

polytomy within the relationships of this clade (Fig. 1), this charaeter state might be plesiomorphic, because *P. enaulus, P. merus, P. sprousei*, and *P. mysticus* have the triangular embolus pointing diagonally to the longitudinal axis of the femur (character state 0) (Fig. 8), which could be eonsidered a derived state. This is the same for *P. hoogtraali* + *P. pedregosus* that have an embolus that is triangular dorsally and rounded ventrally (derived state) (char. 39, character state 5) (Valdez-Mondragón 2010; Figs. 58, 93). In species with an apical triangular embolus, the apical ventral coneavity on the embolus (char. 41) (right arrow, Fig. 8) in *P. enaulus, P. merus, P. sprousei, P. tanneri, P. mysticus, P. marialuisae* and *P. michoacanus* seems to have evolved several times convergently (Fig. 1). The third synapomorphy that seems to support the clade is eharacter 51, the curved dorsal projection on the embolus (Valdez-Mondragón 2010; Fig. 10); however, this character has been lost several times (*P. enaulus, P. merus, P. sprousei*, and *P. hoogtraali* + *P. pedregosus*) (Fig. 8). The fourth synapomorphy is the position of the sclerotized cones of the male chelicerae (char. 24), although most of the species have cones on the basal half and the prolateral part of the chelicerae and lateral apophysis (character state 0) (Valdez-Mondragón 2010; Fig. 29, 70, 105). The plesiomorphic state or ancestral state seems to have cones on the basal half and on the prolateral part of the chelicerae and lateral apophysis, leaving a basal zone on the prolateral part without cones (character state 1), which is present on *P. cornutus* and *P. rothi* (Valdez-Mondragón 2010; Figs. 15, 183). The derived character state consists of cones on the prolateral part and toward the prolateral part of the lateral apophysis, leaving an area of half-moon shape without cones between them (character state 3) on *P mysticus* and *P. reddelli* (Fig. 9); and cones scattered throughout the chelicerae (character state 4) appearing twice convergently on *P. franckei* and *P. marialuisae* (Valdez-Mondragón 2010; Figs. 119, 161).

Finally, the only clade supported with high Jackknife and Bremer values is *P. pedregosus* and *P. hoogstraali*, with 76% and 3 respectively. The characters that support this close relationship include the epigynum with lateral median protuberances (char. 14) (Valdez-Mondragón 2010; Figs. 60, 95) and the dorsally triangular and ventrally rounded embolus (char. 39, character state 5) (Valdez-Mondragón 2010; Figs. 58, 93).

Biogeography.—Analyzing the distribution of the two species groups, I note that the *globosus* group has a distribution in the Mesoamerican and Mexican Mountain biotic components, following the biogeographical scheme of Mexico (Morrone 2004, 2005) (Fig. 22), whereas the *dugesi* group is distributed in the Mesoameriean and Continental Nearctic components (Fig. 23). The biotic components are defined by taxa with a common history, which form biogeographical patterns (Morrone 2005). The biogeography of Mexico is extremely complex; there were several dispersal and vicariance events because Nearctic and the Neotropical biotic elements, known as the Mexican Transition Zone, overlap in Mexico, (Morrone 2005; Brooks 2005). Halffter et al. (1995). Halffter (2003) reviewed this condition, working with the insects of the region.

The Mexican Transition Zone is geographically delimited by the Transmexican Volcanic Belt, a mountain complex in central Mexico (states of Guanajuato, Estado de México, Distrito Federal, Jalisco, Michoacán, Puebla, Oaxaca, Tlaxcala, and Veracruz) (Morrone 2006). By analyzing the distribution of the *globosus* and *dugesi* species groups, I determined that the *globosus* group has a natural distribution primarily toward the south of the Transmexican Volcanic Belt (Neotropical region) (Fig. 22), while the *dugesi* group has a natural distribution toward the north of the Transmexican Volcanic Belt (Nearctic region) (Fig. 23). Given the complex biogeography in Mexico, apparently a large-scale vicariant event separated the two major clades within the genus *Physocyclus* (Fig. 1).

In conclusion, although the genus *Physocyclus* is monophyletic, as are the two species groups within, numerous internal polytomies, mostly within the *dugesi* group, blur a clear phylogenetic picture at the speeies level. Future studies should use new evidence and add molecular data to help resolve the relationships among the species.

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Appendix.—Character matrix used in the phylogenetic analyses, composed of 33 taxa (29 ingroup, 4 outgroup), and 54 morphological characters (44 binary and 10 multistate).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Priscula binghamae	1	1	0	0	0	0	0	0	-	0	0	0	0	0	0	0	-	-	0	0	0	0	-	-	0	0	0	1	0	0	0	0	0	0	0	1	0	-	8	-	-	-	0	-	-	1	1	0	0	1	0	-	0	0
Artema atlanta	1	0	0	0	0	0	0	0	-	3	0	2	0	0	0	1	3	3	0	0	0	0	-	-	0	0	0	0	1	0	0	0	0	0	0	1	0	-	7	-	-	-	0	-	0	0	0	0	0	0	0	-	0	1
Trichocyclus nigropunctatus	0	1	0	0	0	0	0	0	-	5	0	0	0	0	0	0	-	-	1	1	0	0	-	-	0	0	0	0	1	0	0	0	0	0	0	0	0	-	6	-	-	-	0	-	0	2	0	1	1	0	0	-	0	0
Trichocyclus nullarbor	0	1	0	0	0	0	0	0	-	5	0	0	0	0	0	0	-	-	1	1	0	0	-	-	0	0	0	0	1	0	0	0	0	0	0	0	0	-	6	-	-	-	0	-	0	2	0	1	1	0	0	-	0	0
P. huacana	1	1	1	1	0	1	1	1	0	2	0	1	0	0	1	1	1	2	0	1	0	0	-	-	0	0	0	0	1	1	0	1	0	0	0	1	1	2	0	-	-	-	0	-	0	1	0	0	0	0	0	-	1	0
P. montanoi	1	1	1	1	0	0	1	1	0	2	0	1	0	0	1	1	2	2	0	1	1	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	4	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. modestus	1	1	0	0	0	0	1	1	0	2	1	1	0	0	1	1	2	2	0	1	1	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	3	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. sarae	1	1	0	0	0	0	1	1	0	2	1	1	1	0	1	1	2	2	0	1	1	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. globosus	1	1	1	1	0	0	1	1	0	2	0	1	1	0	1	1	1	9	0	1	0	1	0	-	1	0	0	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. guanacaste	1	1	1	1	0	0	1	1	0	2	0	1	1	0	?	1	1	9	0	1	0	1	0	-	1	0	0	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. validus	1	1	1	1	0	0	0	1	0	2	1	1	1	0	1	1	1	6	0	1	0	0	-	-	0	0	0	0	1	0	0	0	0	0	1	1	1	1	2	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. paredesi	1	1	1	1	0	0	1	1	0	2	1	1	1	0	0	1	0	7	0	1	0	0	-	-	0	0	1	0	1	1	0	0	0	0	1	1	1	1	0	-	-	-	0	-	0	1	0	0	0	0	0	-	1	0
P. lautus	1	1	1	1	0	1	1	1	0	2	1	1	1	0	?	1	0	5	0	1	0	0	-	-	0	0	1	0	1	1	0	0	0	0	1	1	1	0	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. bicornis	1	1	0	0	0	1	1	1	0	2	0	1	1	0	0	1	1	6	0	1	0	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. gertschi	1	1	0	0	0	1	1	1	0	2	1	1	0	0	?	1	0	7	0	1	0	0	-	-	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0	-	-	-	0	-	0	1	0	0	0	0	0	-	0	0
P. platnicki	1	1	0	0	0	1	1	1	1	1	0	1	0	0	1	1	4	8	0	1	0	1	0	-	0	0	1	0	1	0	0	0	0	0	0	1	0	-	1	-	-	-	1	1	1	1	0	0	0	0	0	-	0	1
P. cornutus	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	1	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	-	3	-	-	-	1	1	1	1	0	0	0	0	0	-	0	1
P. rothi	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	0	4	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	-	3	-	-	-	1	1	1	1	0	0	0	0	0	-	0	1
P. franckei	1	1	0	0	0	0	1	1	1	4	0	1	0	0	1	1	0	4	0	1	0	1	1	4	0	0	0	0	1	0	1	0	0	1	1	1	0	-	4	0	0	-	1	4	0	1	0	0	0	0	0	-	0	1
P. californicus	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-	1	0	1	1	0	0	0	0	1	1	0	1
P. enaulus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	0	1	0	1	1	0	0	0	0	0	-	0	1
P. merus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	0	1	0	1	1	0	0	0	0	0	-	0	1
P. tanneri	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	1	2	1	0	1	1	0	0	0	0	1	0	0	1
P. brevicornus	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-	1	0	1	1	0	0	0	0	1	1	0	1
P. sprousei	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	0	1	0	1	0	1	1	0	0	0	0	0	-	0	1
P. dugesi	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	1	1	2	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-	1	0	1	1	0	0	0	0	1	0	0	1
P. darwini	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	0	0	1	0	1	1	2	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	0	-	1	0	1	1	0	0	0	0	1	0	0	1
P. reddelli	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	0	1	0	1	1	3	0	1	0	0	1	0	1	0	0	1	1	1	0	-	4	1	0	-	1	0	1	1	0	0	0	0	1	0	0	1
P. mysticus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	-	-	0	1	0	1	1	3	0	1	0	0	1	0	0	0	1	1	1	1	0	-	4	0	1	1	1	3	1	1	0	0	0	0	1	0	0	1
P. marialuisae	1	1	0	0	1	0	1	1	1	0	0	1	0	0	1	0	-	-	0	1	0	1	1	4	0	0	0	0	1	0	0	0	0	1	1	1	0	-	4	1	1	1	1	6	1	1	1	0	0	0	1	0	0	1
P. michoacanus	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	4	1	1	1	1	2	1	1	0	0	0	0	1	0	0	1
P. hoogstraali	1	1	0	0	0	0	1	1	1	0	0	1	0	1	1	1	1	1	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	5	-	-	-	1	2	1	1	0	0	0	0	0	-	0	1
P. pedregosus	1	1	0	0	0	0	1	1	1	0	0	1	0	1	1	1	1	1	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	-	5	-	-	-	1	5	1	1	0	0	0	0	0	-	0	1

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Three new Peruvian species of *Protimesius* (Opiliones: Laniatores: Stygnidae)

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Abstract. Three new species of the harvestmen genus *Protimesius* Roewer 1913 are described from the Amazonian region of Peru. *Protimesius amigos* n. sp. from Madre de Dios Department may be distinguished by the absence of an anterior prominence on the prosoma and the presence of five pairs of basal large setae on the penis. *Protimesius machiguenga* n. sp. and *P. kakinte* n. sp. are described from the Lower Urubamba region of Cusco Department; *P. machiguenga* n. sp. is similar to *P. cirio* Villarreal-Manzanilla & Pinto-da-Rocha 2006 and can be distinguished from it by the presence of a conspicuous dorsal prolateral row of tubereles on the male tibia IV; *P. kakinte* n. sp. is similar to *P. amphichelis* Roewer 1931 and *P. boibumba* Villarreal-Manzanilla & Pinto-da-Rocha 2006, but can be separated from these by the development of tubercles on the femur and tibia IV, and by the number of retrolateral tubercles on male femur IV. New records of *P. albilineatus* (Roewer 1957) from Loreto department in northern Peru are also reported.

Keywords: Amazonia, Opiliones, Peru, *Protimesius*, Stygninae

The harvestman family Stygnidae is distributed throughout the central-northern portion of South America and Lesser Antilles and consists of 98 species (Pinto-da-Rocha 2000; Villarreal-Manzanilla & Pinto-da-Rocha 2006; Pinto-da-Rocha 2007; Hara & Pinto-da-Rocha 2008; Kury & Pinto-da-Rocha 2008; Kury 2009; Pinto-da-Rocha & Carvalho 2009; Pinto-da-Rocha & Tourinho 2012; Bragagnolo 2013). The family is divided into three subfamilies: Nomoclastinae, Heterostygninae, and Stygninae (Pinto-da-Rocha 2007).

The Neotropieal genus *Protimesius* Roewer 1913 includes moderate to large-bodied stygnine harvestmen, easily diagnosed by the elongated coxa to patella of the pedipalp. The two synapomorphic characters of the genus (Pinto-da-Rocha & Villarreal-Manzanilla 2009) are the bifid tibial anterior sockets of the pedipalps (see Pinto-da-Rocha 1997, Figs. 584 & 585) and the scopula of tarsi III–IV with long hairs having thin apices (see Pinto-da-Rocha 1997, Fig. 595). The relationships within the genus are unclear, according to Pinto-da-Rocha & Villarreal-Manzanilla (2009). *Protimesius gracilis* Roewer 1913 has been recognized as the sister species of two other clades of species, one consisting of *P. laevis* Soerensen 1932 and *P. cirio* Villarreal-Manzanilla & Pinto-da-Rocha 2006 and the other including six species (*P. trocaraincola* Pinto-da-Rocha 1997, *P. baliensis* Pinto-da-Rocha & Villarreal-Manzanilla 2009, *P. amplichelis* Roewer 1931, *P. evelineae* (Soares & Soares 1978), *P. foliadereis* Villarreal-Manzanilla & Pinto-da-Rocha 2006 and *P. boibumba* Villarreal-Manzanilla & Pinto-da-Rocha 2006). Seven other previously known species of the genus form a polytomy with these two elades (Pinto-da-Rocha & Villarreal-Manzanilla 2009).

A new phylogenetic hypothesis of the species of *Protimesius* was recently proposed with several changes of the relationships within the genus; however, most clades are supported by only one synapomorphy, and the main characters used to separate species are very homoplastic (Bragagnolo 2013). A reanalysis of *Protimesius* phylogeny, including more characters, is necessary to resolve this controversy.

Protimesius, currently consisting of 20 species, is restricted to Brazil, Ecuador, and Peru (Pinto-da-Rocha & Villarreal-Manzanilla 2009; Bragagnolo 2013). Species of this genus inhabit the northern Atlantic rainforest, Amazonian lowland, and Andean rainforests. *Protimesius* has been previously reported from Peru represented by a single species, *P. albilineatus* (Roewer 1957) from the Ucayali and Madre de Dios departments (Roewer 1957; Pinto-da-Rocha 1997; Kury 2003). During the last 10 years, we have conducted several field expeditions in Peru, including the rainforests of the Amazonian region. In the present contribution, we describe three new Peruvian species of *Protimesius* and report new records for *P. albilineatus*. The description of these three new speeies increases the number of named species in the genus to 23, with four occurring in Peru.

METHODS

The specimens examined for this study are deposited in the collections of Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Cusco, Peru (MHNC) and Museu de Zoologia da Universidade de São Paulo, Brazil (MZSP). Specimens were collected on vegetation at night by ultraviolet (UV) light detection. The integument, mainly of the tergites, sternites, and articular membranes of legs, is slightly fluorescent under ultraviolet light. Morphological terminology follows Pinto-da-Roeha (1997). All measurements are given in millimeters and were obtained following the methodology of Acosta et al. (2006). We recorded measurements and produced illustrations using a Leica MZ-APO stereomicroscope fitted with an ocular micrometer and a camera lucida. Distribution maps were generated using DIVA-GIS Version 5.4 (http://www.diva-gis.org/) by superimposing geo-referenced point locality records on a digital elevation dataset from the CGIAR Consortium for Spatial Information (CGIAR-CSI) available at http://srtm.csi.cgiar.org.

TAXONOMY

Family Stygnidae Simon 1879
Protimesius Roewer 1913

Protimesius Roewer 1913:439.

Type species.—*Protimesius gracilis* Roewer 1913 by monotypy.

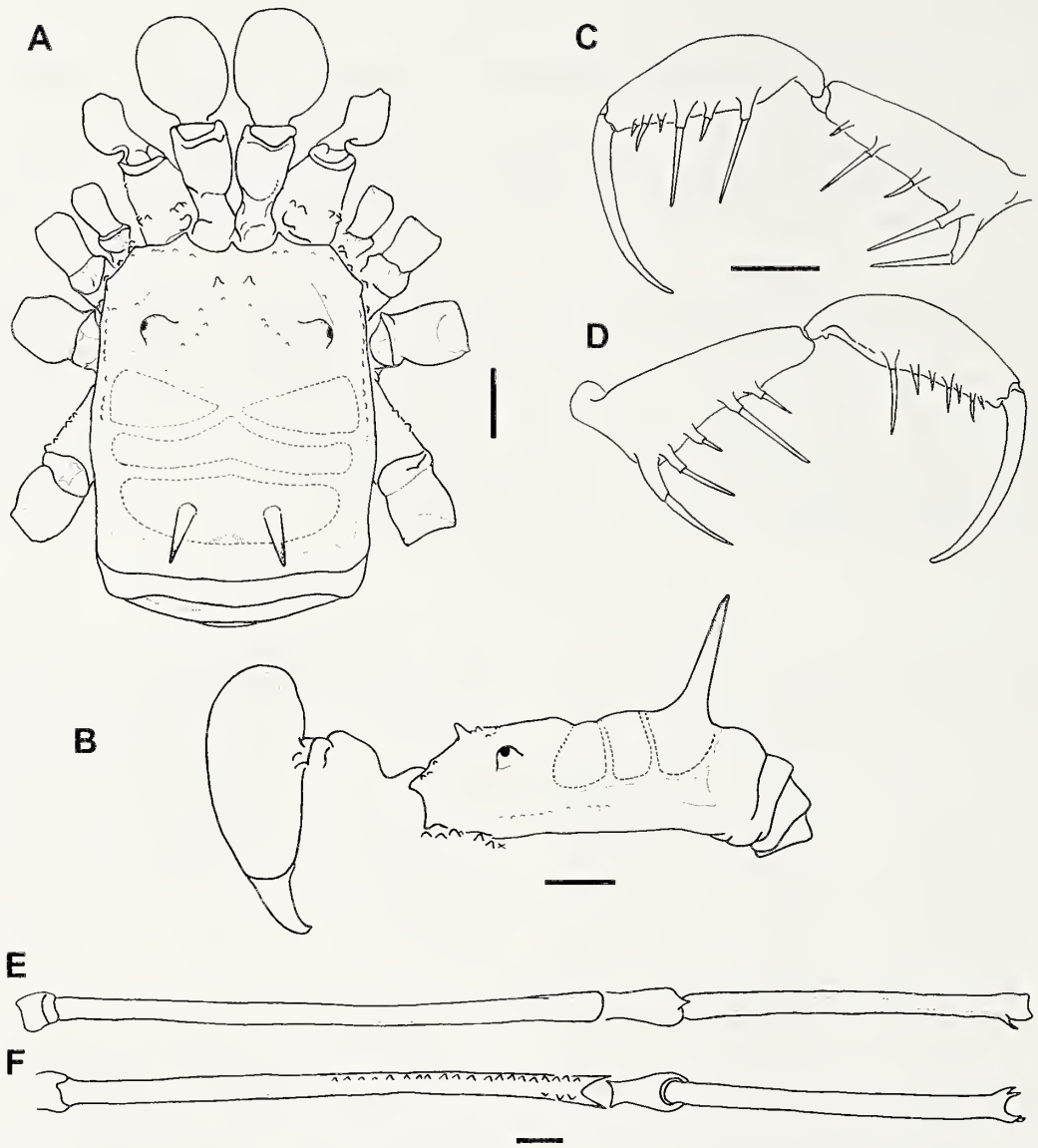

Figure 1.—*Protimesius amigos* new species: Paratype male (MHNC). A, B. Habitus: A. Dorsal view; B. Lateral view. C, D. Right pedipalp: C. Mesal view; D. Ectal view. E, F. Leg IV: E. Dorsal view; F. Ventral view. Scale bars: 1 mm.

Protimesius amigos new species
Figs. 1A–F, 4E & F, 5

Type material.—PERU: *Madre de Dios Department*, Manu Province, Manu District: Holotype male (MHNC), CICRA, Los Amigos Biological Station, confluence between Los Amigos and Madre de Dios rivers, 12°34′07″S, 70°05′57″W, 270 m, 12 December 2005, J.A. Ochoa, collected at night with UV light. Paratypes: 1 male, 1 female, collected with holotype (MHNC); 1 male, 1 female, collected with holotype (MZSP 36823).

Etymology.—The specific name in a noun in apposition taken from the Spanish word *amigos*, meaning "friends," and refers the type locality, Los Amigos River.

Diagnosis.—*Protimesius amigos* is most similar to *P. albilineatus*, based on the similar ornamentation on male leg IV (Fig. 1E, F), two ventral rows of small tubercles on the distal half of femur IV, tibia IV smooth or without conspicuous rows of tubercles, and the ventral plate of the penis with lateral margins concave (Figs. 4E, F). *Protimesius amigos* may be separated from this species by the absence of an anterior prominence on the prosoma, which is well developed in *P. albilineatus*. The penis in *Protimesius amigos* possesses five pairs of basal large setae and with a concave distal margin of the ventral plate, compared to *P. albilineatus*, with four pairs of basal setae and a straight distal margin of ventral plate.

Description.—*Male (holotype): Measurements*: Dorsal scutum length 4.86; prosoma length 2.50; dorsal scutum width 3.86; prosoma width 3.71; interocular distanee 2.50; chelicera: II 3.29, III 1.79; pedipalp 19.0; leg I 20.5, II 38.0, III 29.0, IV 38.0.

Dorsum (Fig. 1A, B): Anterior margin of earapace with some small granules. Prosoma without anterior prominence, two tubercles anteriorly, and several sparse small granules medially. Eye mounds smooth. Interocular region with several

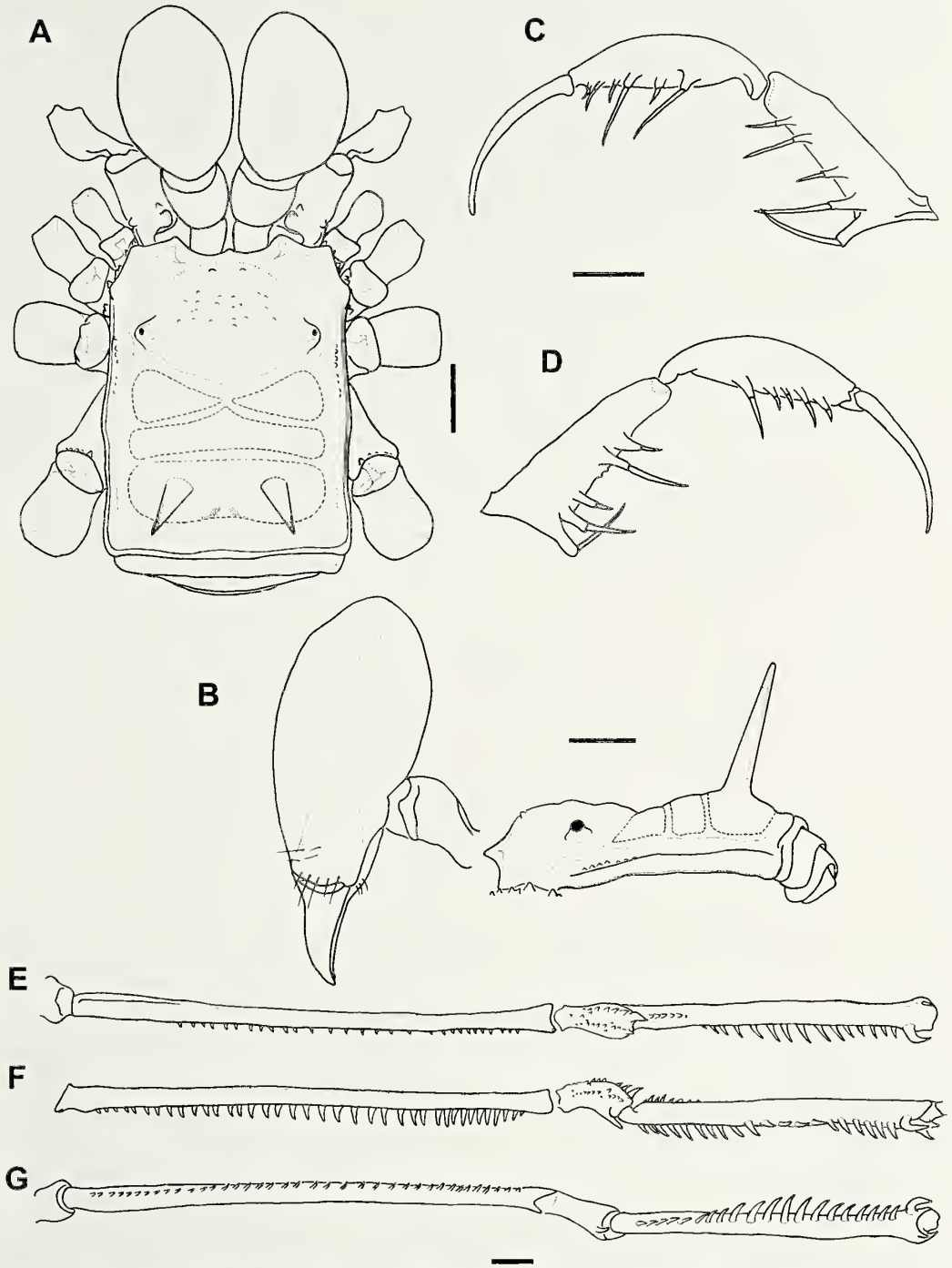

Figure 2.—*Protimesius machiguenga* new species: Holotype male (MHNC). A, B. Habitus: A. Dorsal view; B. Lateral view. C, D. Right pedipalp: C. Mesal view; D. Ectal view. E–G. Leg IV: E. Dorsal view; F, Retrolateral view; G. Ventral view. Scale bars: 1 mm.

granules. Lateral margins with 7-7 tubercles from coxa III to coxa IV (one paratype possesses 9–10, Fig. 1A). Area I–III without tubercles; I divided, III with two large spines, divergent slightly toward posterior. Posterior margin and free tergites smooth.

Venter: Coxa I with a medial row of 7-7 tubercles, two small anterior, 4-3 small posterior, three apical (anterior largest); II with medial row of 11–10 tubercles, three small anterior, 7-6 small posterior, three apical; III with a weak row of 9–10 small tubercles, 7-6 small posterior scattered tubercles, and four apical tubercles; IV with scattered small tubercles. Genital operculum with 8 granules. Free sternites smooth.

Chelicerae: Swollen. Segment I smooth; II with one basal elongated, three small teeth, with several setae near the base of finger; III with one elongated median and two small subdistal teeth.

Pedipalp: Coxa with one large dorsobasal, 5–6 dorsosubbasal, and four ventral tubercles. Trochanter with four ventral tubercles, one basal, one medial (the largest), and two small distal. Femur with one ventrobasal tubercle; patella smooth; tibia, mesal IIiIi; ectal IIiIi; tarsus, mesal IiIiii; ectal Iiiiii.

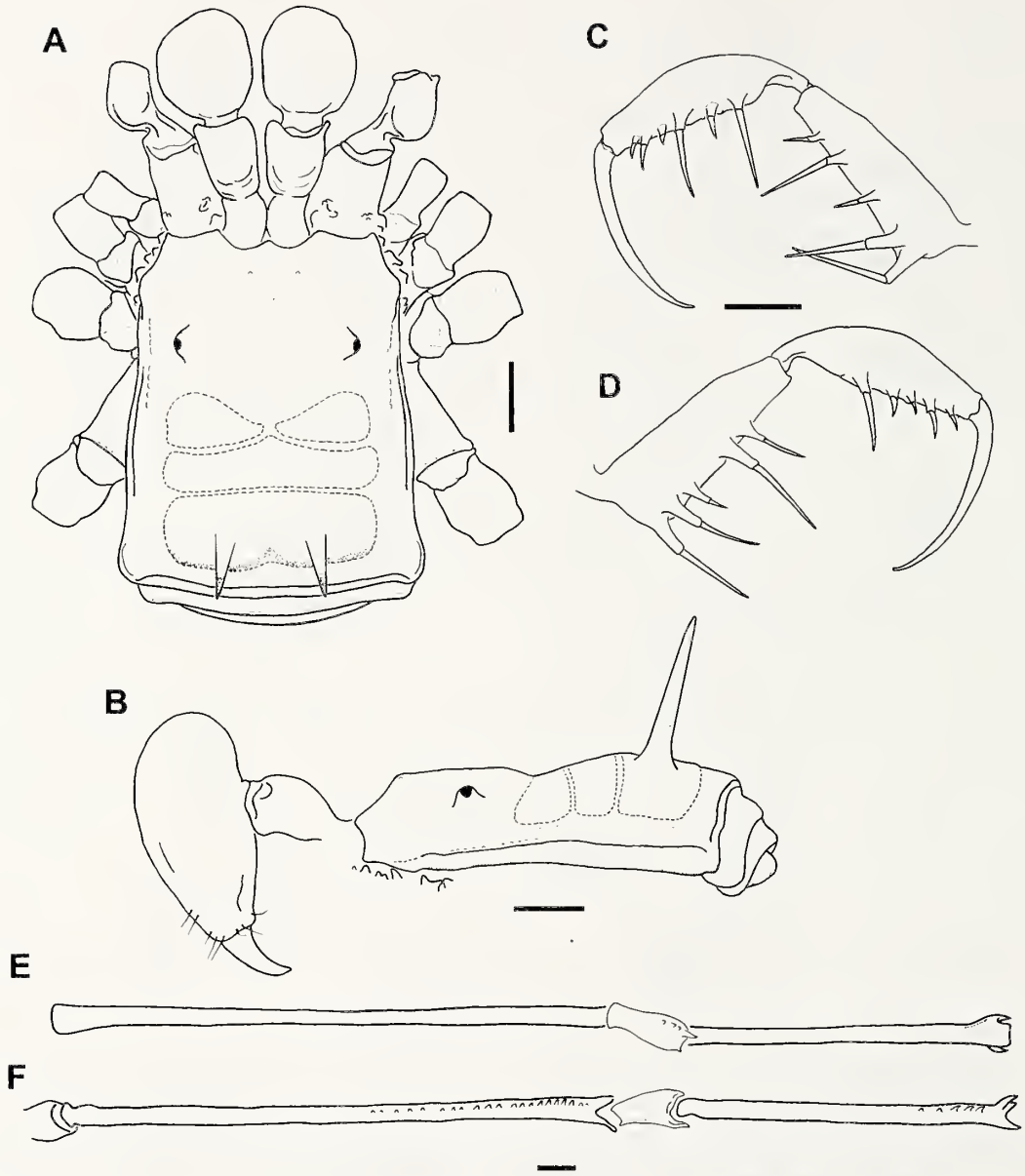

Figure 3.—*Protimesius kakinte* new species: Holotype male (MHNC). A, B. Habitus: A. Dorsal view; B. Lateral view. C, D. Right pedipalp: C. Mesal view; D. Ectal view. E, F. Leg IV: E, Dorsal view; F, Ventral view. Scale bars: 1 mm.

Legs: Coxa I with three dorsal tubercles, the anterior smaller, the posterior bifid; II with three dorsal, the posterior fused with coxa III; III with one tubercle, IV with five dorsal small granules distally and some tubercles laterally. Trochanters I–IV with three ventral tubercles, antero-distal smaller in I–II or vestigial in III and IV. Femur IV (Fig. 1E, F) with two ventral rows of small tubercules on distal half, prolateral row with three tubercles, retrolateral with 21, progressively increasing in size distally, except the last one, which is smaller. Patella IV (Fig. 1E, F), ventral side smooth, dorsal side with a conspicuous distal tubercle. Tibia IV smooth without tubercles, prolaterodorsal and retrolateral distal spines moderate developed. Tarsal segmentation: 7, 13, 6, 7.

Penis (Figs. 4E, F)*:* Truncus with five pairs of basal large setae; ventral plate lateral and distal sides concave; with three pairs of distal large setae and without intermediary setae. Glans with dorsal process well developed, lower than stylus; stylus apex swollen and without small subapical setae.

Color: Yellowish brown; chelicerae, prosoma, coxa of pedipalps and legs, and scutum slightly darker; with well developed reticulate pigmentation especially in antero-lateral parts of prosoma, lateral borders of scutum and area III; free tergites and sternites reddish brown with dark reticulate pigmentation; legs with brown reticulate pigmentation. Membrane between posterior margin and free tergite I white.

Female (paratype, MHNC): Measurements: Dorsal scutum length 4.36; prosoma length 1.86; dorsal scutum width 3.79; prosoma width 3.64; interocular distance 2.36; chelicera: II 2.21, III 1.50; pedipalp 20.86; leg I 22.0, II 40.5, III 31.0, IV 41.0.

Somatic morphology: Similar to male, differs in the following features: reticulate pigmentation more evident than male, especially in prosoma and free tergites and sternites.

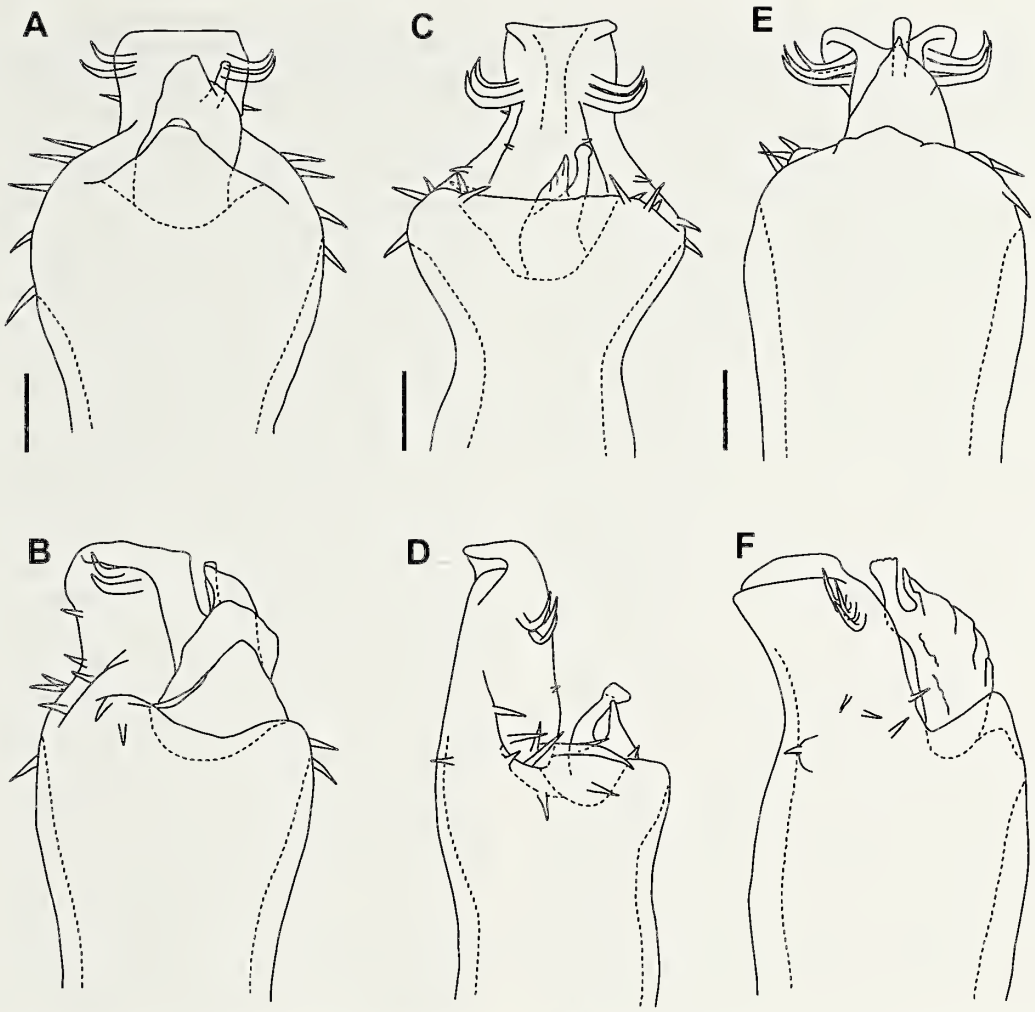

Figure 4.—Distal portion of penis of Peruvian *Protimesius* spp.: A, B. *P. kakinte* new species. A, dorsal view; B, lateral view. C, D. *P. machiguenga* new species. C, dorsal view; D, lateral view. E, F. *P. amigos* new species. E, dorsal view; F, lateral view. Scale bars: 0.1 mm.

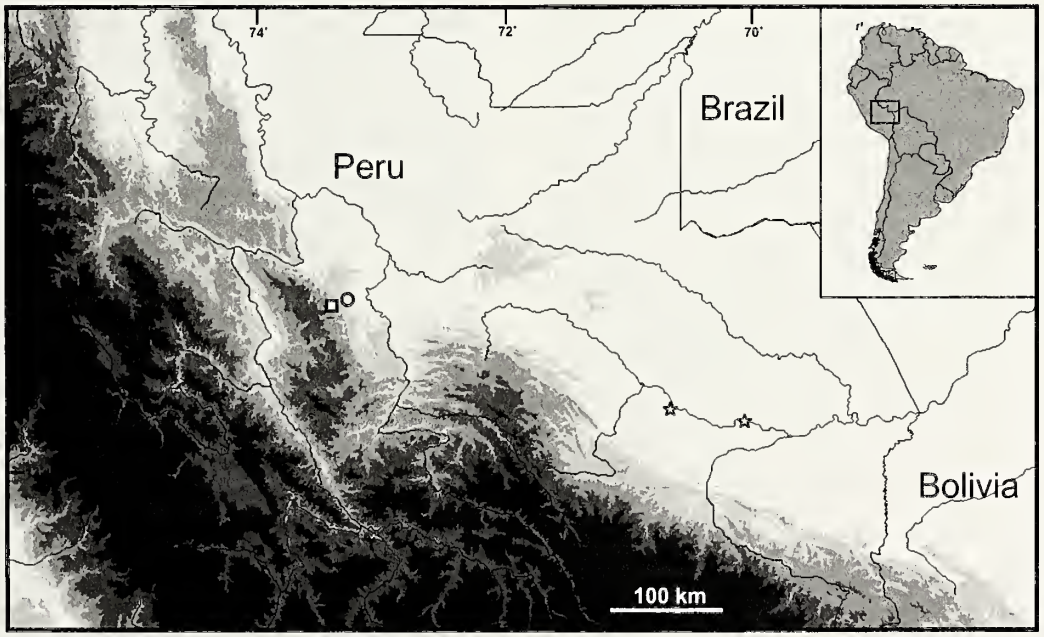

Figure 5.—Map of the known locality records of *Protimesius* spp. in southeastern Peru. *P. amigos* new species (stars), *P. machiguenga* new species (square), *P. kakinte* new species (circle).

Chelicerae slightly less swollen; prosoma less granular than male; leg IV: femur, patella and tibia smooth, without tubercles. Tarsal segmentation: 7, 14, 6, 7. Pedipalpal tarsal formula; mesal IiIiii, ectal Iiiii.

Distribution.—Peru: Madre de Dios.

Protimesius machiguenga new species
Figs. 2A–G, 4C & D, 5

Type material.—PERU: *Cusco Department*, La Convención Province, Echarati District: Holotype male (MHNC), Kiñancaroni, Reserva Comunal Machiguenga, between Taini and Kitepampani, near the confluence between Ayeni and Yari Rivers, 11°36′44″S, 73°23′18″W, 497 m, 18 October 2006, J.A. Ochoa, collected at night with UV light. Paratypes: 1 female, collected with holotype (MHNC), 1 male, 1 female, collected with holotype (MZSP 36821).

Etymology.—The specific name is a noun in apposition and refers to the Reserva Comunal Machiguenga, an area protected by the Peruvian government, located on the eastern slopes of the Vilcabamba mountain range in Cusco Department, where this species occurs.

Diagnosis.—*Protimesius machiguenga* appears to be closely related to *P. cirio* and *P. boibumba*, based on the presence of ventral row of tubercles on male femur IV. *Protimesius machiguenga* may be separated from one another by the number of tubercles on femur IV; it consists of 37–38 tubercles (Fig. 2E) in *P. machiguenga*, 24 in *P. cirio,* 46 in *P. boibumba*; additionally tibia IV in *P. machiguenga* possesses a conspicuous dorsal prolateral row of tubercles and one retrolateral row of small tubercles, whereas in *P. cirio* the tibia IV is smooth, and *P. boimbumba* has only a ventral row of tubercles. The shape of the penis of *Protimesius machiguenga* (Fig. 4C, D) differs from that of *P. cirio* by the presence of three pairs of distal setae (also present in *P. boibumba)* and dorsal process well developed, compared with 4–5 pairs of distal setae and absence of dorsal process in *P. cirio*.

Description.—*Male (holotype): Measurements*: Dorsal scutum length 5.14; prosoma length 2.43; dorsal scutum width 4.36; prosoma width 4.14; interocular distance 2.79; chelicera: II 5.07, III 2.43; pedipalp 24.2; leg I 28.0, II 54.5, III 39.0, IV 50.5.

Dorsum (Fig. 2A, B)*:* Anterior margin of carapace smooth. Prosoma without anterior prominence, two moderate tubercles anteriorly, and several sparse, small granules medially. Eye mounds smooth. Interocular region smooth. Lateral margins with 7–8 small tubercles from coxa III to anterior margin of coxa IV. Area I–III without tubercles; I divided; III with two large spines, divergent slightly toward posterior. Posterior margin and free tergites finely granulated.

Venter: Coxa I with a medial row of 7–5 tubercles, two anterior, three apical (anterior largest); II with medial row of 7–8 small tubercles, two apical; III with a weak row of small tubercles, and two apical tubercles; IV smooth. Genital operculum with five granules, three anterior largest. Free sternites smooth.

Chelicerae: Strongly swollen. Segment I smooth; II with one basal elongated and three medial small teeth, with several setae near the base of finger; III with one elongated median and two small subdistal teeth.

Pedipalp: Coxa with one large dorsobasal, two dorsosubbasal, and three ventral tubercles, distal largest. Trochanter with four ventral tubercles, one basal, one medial (the larger), and two distal. Femur with one ventrobasal tubercle, patella smooth. Tibia: mesal IIiIi, ectal IIiIi. Tarsus: mesal IiIiii, ectal IiiIi.

Legs: Coxa I with three dorsal tubercles, the anterior smaller, the posterior bifid; II with 2–3 dorsal (a small additional granule could be present), the posterior bifid and fused with coxa III; III with one tubercle; IV with one prominent and three small granules distally. Trochanters I–IV with three ventral tubercles, antero-distal smaller in I–III or vestigial in IV. Femur IV (Fig. 2E–G) with a ventral row of 37–38 tubercles, progressively increasing in size distally, except the last three, which are smaller. Patella IV (Fig. 2E–G): dorsal side with one dorsoprolateral row of seven tubercles, progressively increasing in size distally; one retrolateral row of small tubercles; and four dorsal small granules; ventral side with a conspicuous distal tubercle. Tibia IV (Fig. 2E–G) with one dorsal row of seven proximal small tubercles; one ventral row of 20–23 conspicuous tubercles, medial tubercles slightly tortuous retrolaterally and much larger than basal and distal one; prolaterodorsal and retrolateral distal spines well developed. Tarsal segmentation: 8, 19, 6, 7.

Penis (Figs. 4C, D)*:* Truncus with 10 pairs of large basal setae; ventral plate pentagonal, distal margin straight; with three pairs of distal large setae and one pair of intermediary setae smaller than others and placed more dorsally than distal one. Glans with dorsal process well-developed, lower stylus; stylus apex swollen and with small subapical setae.

Color: Prosoma, pedipalps and legs I–III, yellowish brown with slight reticulate pigmentation; antero-lateral parts of prosoma, lateral borders of scutum and area III with dark reticulate pigmentation; free tergites and sternites brown; chelicerae brown with reticulate pigmentation; leg IV reddish brown.

Female (paratype, MHNC): Measurements: Dorsal scutum length 5.0; prosoma length 2.14; Dorsal scutum width 4.29; prosoma width 3.71; interocular distance 2.29; chelicera: II 2.14, III 1.50; pedipalp 23.4; leg I 26.0, II 51.5, III 38.0, IV 51.5.

Somatic morphology: Similar to male, differs in the following features: chelicerae and prosoma slightly darker, especially in borders; free tergites and sternites with well developed brown pigmentation. Chelicerae short, not swollen; prosoma less granular than male. Leg IV: femur, patella and tibia smooth, without tubercles. Tarsal segmentation: 7, 18, 6, 7.

Distribution.—Known only from the type locality.

Protimesius kakinte new species
Figs. 3A–F, 4A & B, 5

Type material.—PERU: *Cusco Department*, La Convención Province, Echarati District: Holotype male (MHNC), Sariteto, Kitepampani, Ayeni River near the Reserva Comunal Machiguenga, 11°35′10″S, 73°20′32″W, 447 m, 17 October 2006, J.A. Ochoa, collected at night with UV light.

Etymology.—The specific name, a noun in apposition, refers to the geographic distribution of this species in Kitepampani, a Kakinte Native Community. Kakinte is a small Amazonian tribal group of southeastern Peru. The Kakinte language belongs to the Arawak language family and is related with the Machiguenga group.

Diagnosis.—*Protimesius kakinte* appears to be most similar to *P. anuplichelis* and *P. boibumba*, based on the presence of

ventral retrolateral row of tubercles on the male femur IV, and ventral retrolateral row on the apex of tibia IV (Fig. 3E–F). *Protimesius boibunba* differs from *P. kakinte* by its well-developed large tubercles on the femur and tibia IV, which are present on the distal two thirds of femur IV and entirely on tibia IV, whereas in *P. kakinte* the tubercles are present on the distal half of femur IV, restricted to five small tubercles distally. *P. kakinte* may be separated from *P. amplichelis* by the number of retrolateral tubercles on femur IV: *P. kakinte* possess 21 tubercles compared to *P. amplichelis* with 9 tubercles; additionally, *P. amplichelis* present a low anterior eminence on the prosoma, which is absent in *P. kakinte*.

Description.—*Male (holotype): Measurements*: Dorsal scutum length 4.79; prosoma length 2.14; Dorsal scutum width 4.0; prosoma width 3.71; interocular distance 2.29; chelicera: II 3.29, III 1.86; pedipalp 22.4; leg I 24.0, II 46.5, III 36.5, IV 47.0.

Dorsum (Figs. 3A, B)*:* Anterior margin of carapace smooth. Prosoma without anterior prominence, two small tubercles anteriorly, and few sparse small granules medially. Eye mounds smooth. Interocular region smooth. Lateral margins with 10-10 small tubereles from coxa III to anterior margin of coxa IV. Area I–III without tubercles; I divided; III with two large spines, divergent slightly towards posteriorly. Posterior margin and free tergites smooth.

Venter: Coxa I with a medial row of 6-6 tubercles, one small anterior, 3–4 small posterior, three apical; II with a weak medial row of 6–5 small tubercles, three apical; III with some small scattered granules and two apical tubercles (anterior vestigial); IV with one apical tubercle. Genital operculum with two weak granules. Free sternites smooth.

Chelicerae: Swollen. Segment I smooth; II with one basal elongated, three small teeth, with some setae near the base of finger; III with one elongated median and two small subdistal teeth.

Pedipalp: Coxa with one large dorsobasal and 4–6 dorsosubbasal tubercles, ventral side with a row of three ventral tubercles (the distal largest) and one posterior small tubercle. Trochanter with one small dorsal and four ventral tubercles, one basal, one medial (the largest), and two small distal. Femur with one ventrobasal tubercle. Patella smooth. Tibia: mesal IIiIi, ectal IIiIi. Tarsus: mesal IiIiii, ectal Iiiii.

Legs: Coxa I with three dorsal tubercles, the anterior smaller, the posterior bifid; II with two dorsal, the posterior bifid and fused with coxa III; III with one tubercle; IV with three dorsal small granules distally. Trochanters I–IV with three ventral tubercles, antero-distal smaller in I–II, vestigial in III–IV. Femur IV (Fig. 3E, F) with a ventral retrolateral row of 21 small tubercles on distal half, progressively increasing in size distally, except the last two, which are smaller. Patella IV (Fig. 3E, F) with a dorsal row of four small tubercles progressively increasing in size distally. Tibia IV (Fig. 3E, F) with a ventral row of five small tubercles distally; prolaterodorsal and retrolateral distal spines moderate developed. Tarsal segmentation: III, 6, IV, 7 (I and II incomplete).

Penis (Figs. 4A, B)*:* Truncus with eight pairs of basal large setae; ventral plate rectangular, distal margin straight; with two pairs of distal large setae and 1 pair of intermediary setae smaller than others and placed more ventrally than distal one. Glans with dorsal process well developed, same height as stylus; stylus cylindrical and without small setae.

Color: Chelicerae, prosoma, Areas I–III, and leg IV, reddish brown; pedipalps and legs I–III yellowish brown; free tergites and sternites brown. Slight reticulate pigmentation on chelicerae, antero-lateral parts of prosoma, lateral borders of scutum and area III; patella, tibia and tarsus of pedipalps and legs I–III with dark reticulate pigmentation.

Female: unknown.

Distribution.—Known only from the type locality.

Protimesius albilineatus (Roewer 1957)

Obidosus albilineatus Roewer 1957:82, Fig. 18.

Protimesius albilineatus: Pinto-da-Rocha 1997: 276, 277, Figs. 357–361, 539, 540, 603; Kury 2003: 230, 268, 282, 285, 286; Pinto-da-Rocha & Villarreal-Manzanilla 2009:55. Fig. 12, table 3; Bragagnolo 2013: 286, Fig. 1.

New material examined.—PERU, Loreto Department, *Maynas, Fernando Lores Province*: Comunidad El Chino, Tahuayo River, 04°18′19″S, 73°13′22″W, 101 m, 25 February 2008, C. Gil and J.A. Ochoa, rainforest, collected at night with UV light, 1 male, 5 females (MHNC), 1 male, 7 females (MZSP 36824), 1 male, 4 females (MZSP 36822).

Distribution.—Brazil: Amazonas; Ecuador: Napo; Peru: Madre de Dios, Ucayali and Loreto.

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Observations on the life history of *Eukoenenia chilanga* Montaño (Arachnida: Palpigradi)

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Abstract. The life history of a palpigrade is reconstructed through morphological and morphometric multivariate analyses applied to a series of 37 individuals collected in a single locality in Tlalnepantla, Estado de México, during different seasons. Those analyses reveal the presence of three life stages: protonymph, deutonymph and adult. Morphologically, males and females can be distinguished as deutonymphs and adults. Morphometrically the sexes can be recognized in all of the life stages, unprecedented for the family Eukoeniidae.

Keywords: Palpigrades, partial life history, sexual dimorphism

Our current knowledge of the life history of palpigrades is quite limited and slightly confusing because of the terminology chosen by former authors. Living members of the order Palpigradi are classified into two different families: Prokoeneniidae Condé and Eukoeneniidae Petrunkevitch, which differ in their life histories. Rucker (1903) presented the first life history of a palpigrade, specifically that of *Prokoeninia wheeleri* (Rucker) from Texas, USA. This species has four life stages, which she named: "First Known Stage", "Second Stage", "Last Stage", and "Adult". Subsequently, Van der Hammen (1982), reviewing Rucker's findings, indicated that the first known instar had no opisthosomatic papillae, two pairs in the second known, and three pairs in the third known instar and in adults. Van der Hammen then speculated that because "There is an important gap between the sizes of Rucker's first and second known instars, and the occurrence of an instar of intermediate size, with one pair of papillae, seems highly probable." Finally, he proposed the existence of a prelarva (without any supporting evidence), a larva (Rucker's first known instar, without papillae), a protonymph (speculative, with one pair of papillae), a deutonymph (Rucker's second instar), a tritonymph (Rucker's third instar) and the adult; thus, from Rucker's four observed instars, Van der Hammen stretched *Prokoenenia* life history to include six stages! Condé (1984) working with *Eukoenenia* proposed that these palpigrades have one instar less than prokoeneniids, but named them as follows: Immature A (sexes not recognizable), Immature B (female subadult), Immature C (male subadult), and adults (male and female); that is, three instars but with different terms between female (B) and male (C) subadults. He further proposed that in prokoeneniids, the Immature A stage is divided into two subsets A_1 and A_2 (Condé 1984, 1996, 1998), which is rather confusing: B and C represent the same instar, whereas A_1 and A_2 represent two consecutive instars.

A preliminary morphological analysis of a series of 12 specimens of *Eukoenenia chilanga* Montaño 2012 collected on 13 June 2003 north of Mexico City suggested the presence of three distinct instars in the field (Table 1; also, see Montaño 2006). Therefore, extra collecting efforts were carried out in 2006–2007, and a comprehensive morphometric analysis was undertaken.

METHODS

The palpigrades were hand collected at Picacho el Jaral, Tlalnepantla, Estado de México, located in the northern reaches of Mexico City (99°56.8886′N, 106°98.888′W, 2345 m). Collection events were restricted to the rainy season (as attempts to locate them during the dry season were unproductive) as follows: 13 June 2003, 13 July 2006, 2 July 2006, 20 November 2006, 6 July 2007, 19 August 2007 and 23 September 2007.

Specimens were fixed in 80% ethanol in the field. They were cleared with lactophenol (50% lactic acid, 25% phenol crystals and 25% distilled water) for about 1 min, and were mounted with Hoyer's (50 ml distilled water, 30 g Arabic gum, 200 g chloral hydrate and 20 ml glycerine) on semi-permanent preparations on individual slides with cover slips. These were dried in an oven for one week at 50 °C, after which the excess Hoyer's liquid was removed with a razor blade, the cover slip was sealed with fingernail polish, and the slide was labeled. The specimens are deposited in the Colección Nacional de Arácnidos (CNAN), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

Specimens were studied under interference phase contrast on a Nikon Optiphot II microscope equipped with an ocular micrometer. The terminology for all structures, including setae, follows Van der Hammen (1989). The characters used for the multivariate analyses were selected on the basis of low perceived deformation on the specimens due to the clearing and mounting technique. A matrix with 27 characters for 34 specimens was produced. The sample contains 12 adults (7 females and 5 males) and 25 immatures, 15 subadults, of which 5 are pre-males (13, 14, 15, 19, 22) and 10 pre-females (16, 17, 18, 20, 21, 23, 24, 25, 26, 27), and 10 nymphs (Appendix 1). The multivariate analyses were performed with the program NTCYSpc version 2.1 (Rohlf 2004), using Principal Component Analysis (PCA), because most of the data are quantitative. PCA graphics were generated showing the dispersion of the operational taxonomic units (OTUs) on the sampling space.

Results from the multivariate analyses were used to calculate "estimated growth ratios" to test for the existence of the presumptive additional instar proposed by Van der

Table 1.—Dates and number of specimens of *Eukoenenia chilanga* collected between 2003 and 2007.

Date	Numbers	Life stages		
		Protonymph	Deutonymph	Adults
13 June 2003	12	2	3	5 ♂, 2 ♀
13 June 2006	5	0	2	2 ♂, 1 ♀
02 July 2006	17	3	3	10 ♂, 1 ♀
20 November 2006	4	1	1	1 ♂, 1 ♀
06 July 2007	19	2	1	4 ♂, 12 ♀
19 August 2007	35	2	8	13 ♂, 14 ♀
23 September 2007	8	0	0	5 ♂, 3 ♀
TOTALS	102	10	18	40 ♂, 34 ♀

Hammen (1982). The growth rate in linear (unidimensional) structures in arthropods is known as Dyar's constant and is the cube root of 2, or 1.26 (Francke & Sissom 1984); thus, if an extra instar occurred between the first and second observed sizes, as hypothesized by Van der Hammen, the growth factor between those first and second observed sizes should be $1.26 \times 2 = 1.59$. The meristic characters (lengths in microns) chosen for these tests were those that showed the highest correlation ($r < 0.96$), and they are basal cheliceral segment, tibia IV, patella IV, basitarsus IV, pedipalp tibia and basitarsus 2 of leg III. The binomial test was used to determine whether the observed frequency of males and females deviated from an expected 50:50 sex ratio (Siegel 1956).

According to Condé's terminology, our "juveniles" correspond to his "A stage", our "subadult females" to the "B stage", and our "subadult males" to the "C stage" in Eukoeneniidae. However, we find the terminology cumbersome and uninformative and propose instead to use the widely accepted "protonymph", "deutonymph (male or female), and "adult".

RESULTS

A total of 102 specimens was collected, 74 adults and 28 immatures, as summarized in Table 1. A detailed morphological analysis of 27 "near-perfect" specimens is summarized in Table 2. Five of the seven characters listed [number of lateral organs, number of deutotritosternal (ep. P) setae, number of lateroventral (lv) setae on sternites X and XI, and number of dorsal (d) setae on tergite XIII] clearly allow the recognition of three distinct morphs, ranked in decreasing size: 1) those with 3 pairs of lateral organs, 5 ep. P, 2 + 2 lv on sternites X and XI, and 2 + 1 + 2 d on tergite XIII; which have developed external genitalia and are clearly adults (Figs. 1 A & B); 2) those with 2 pairs of lateral organs, 3 ep. P, 1 + 1 lv on sternites X and XI, and 1 + 1 + 1 d on tergite XIII; and which have partially differentiated external genitalia and are subadults (Figs. 2 A & B); and finally (3) those with 1 pair of lateral organs, 1 ep. P, no lv seta on sternites X and XI, and 1 + 1 d setae on tergite XIII; and without any modifications on sternites X and XI; i.e., no genitalic differentiation whatsoever (Fig. 2C). Furthermore, on the largest morphs or adults (#1 above), we can recognize two subgroups based on two additional characters in Table 2 [number of ventral (v) setae on sternites X and XI]: 1a) with 5–6 + 0 + 5–6 v setae and 1b) those with 3 + 0 + 3 v setae; which allows the separation of males and females, respectively, without having to examine the genitalia (Figs. 1 C & D). In the last two characters [number of ventral (v) setae on sternites X and XI] adult females resemble subadults (of both sexes), adult males have additional setae, and juveniles have fewer setae. In the intermediate, or subadult, age class, the characters presented in Table 2 do not allow the separation of the sexes, whereas that can be done by examination of the genital plates (Figs. 2 A & B). However, one of the specimens in Table 2, with 2 ep.P setae (usually 1 ep.P in protonymphs and 3 in deutonymphs), and with 2+0+2 v setae on sternite X (usually 2+2 in protonymphs and 3+0+3 in deutonymphs), suggested the possibility of an additional, "rare" instar, between our proposed protonymphs and deutonymphs. Therefore, a Principal Components Analysis of the sizes observed was undertaken to evaluate such a possibility. The results from the PCA analysis are summarized in Table 3, which shows the eigenvalues (right side of the table), and the first component accounts for 88.1% of the variation observed in the sample, and adding the second component raises the value to 90.3%. The eigenvectors (Table 3, left side) show that most values are above 0.9 (or

Table 2.—Results of morphological comparisons in specific structures among palpigrades of different sizes (and age classes) in *Eukoenenia chilanga* from Mexico City (m = adult male, f = adult female, dm = deutonymph male, df = deutonymph female, p = protonymph).

N° of exemplars (Total = 27)	Sex/age	N° of lateral lobes	N° of deuto-tritosternal (*ep.P*) setae	X		XI		XIII
				Ventral		Ventral		Dorsal
				lv	v	lv	v	d
1	m	3	5	2+2	5+0+5	2+2	5+0+5	2+1+2
1	m	3	5	2+2	5+0+5	2+2	6+0+6	2+1+2
2	m	3	5	2+2	6+0+5	2+2	5+0+5	2+1+2
1	m	3	5	2+2	6+0+6	2+2	6+0+5	2+1+2
1	f	3	4	2+2	3+0+3	2+2	3+0+3	2+1+2
2	f	3	5	2+2	3+0+3	2+2	3+0+3	2+1+2
1	f	3	5	2+2	3+0+3	2+2	3+0+3	3+1+3
1	f	3	6	2+2	3+0+3	2+2	3+0+3	2+1+2
6	dm	2	3	1+1	3+0+3	1+1	3+0+3	1+1+1
6	df	2	3	1+1	3+0+3	1+1	3+0+3	1+1+1
1	p	1	2	0	2+0+2	0	2+2	1+1
4	p	1	1	0	2+2	0	2+2	1+1

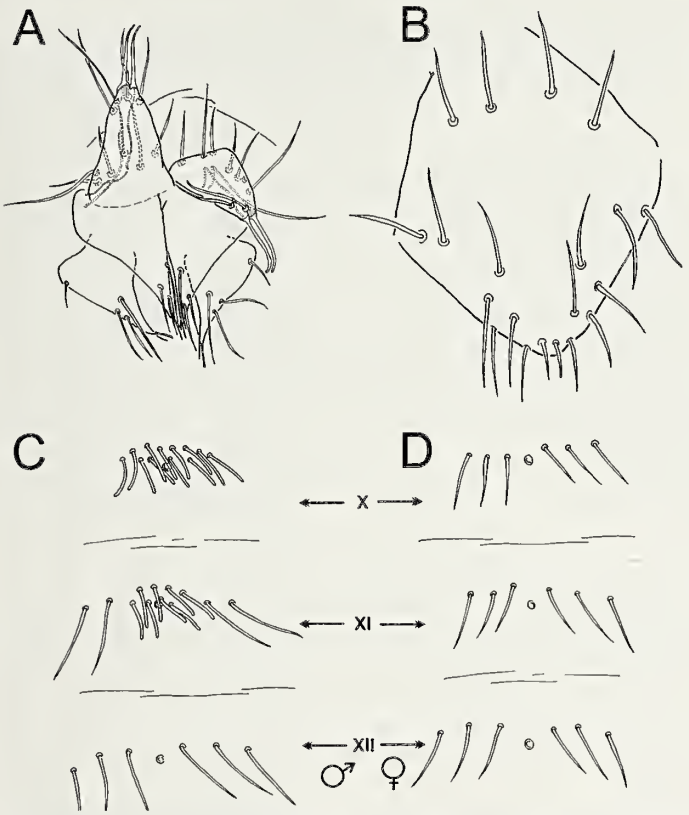

Figure 1.—Sex recognition in adult *Eukoenenia chilanga*, from Tlanepantla, Estado de México. Genital plates. A. Male; B. Female; ventral setae on sternites X, XI and XII; C. Male (X = 6+0+6, XI = 6+0+6, XII = 3+0+3); D. Female (X = 3+0+3, XI = 3+0+3, XII = 3+0+3).

very close to it) in the first component, aceounting for most of the variation observed; those characters are shown in bold type in Table 3. Clearly the most important component in the variation observed is size-related; i.e., the PCA analysis is recognizing distinct instars or age classes in the sample analyzed.

The graph of the dispersion of the samples along the two principal components from the PCA analysis (Fig. 3) shows three distinct size classes on the ordinate, corresponding to the protonymphs, deutonymphs and adults. Thus, one of the specimens in Table 2 (above), despite its variation in two setal counts, is clearly a protonymph with only one pair of lateral organs. Furthermore, the abscissa of the graph shows the morphometric differences due to sexual dimorphism, which manifest as early as in the protonymphs, with females toward the top of the scatter plots, and males lower.

Because of the marked sexual dimorphism in the three life stages observed, we calculated average measurements for six structures separately for males and females and then proceeded to calculate the average growth faetor observed between instars for each sex. The results are shown in Table 4, and in general the growth factors are lower than the expected 1.26, and certainly there are no values approaching the 1.59 growth factor implied by Van der Hammen when he proposed an intermediate, additional instar between the protonymph and the deutonymph. Finally, it is noteworthy that in this species the sex ratio is approximately equal (40 males, 34 females) and does not differ significantly from an expected 50:50 ratio ($z = 0.58$, $P = 0.281$).

DISCUSSION

The life history of eukoeneniids is apparently quite simple and consists of only three active instars: protonymph, deutonymph and adult. Protonymphs (Condé's stage A) cannot be reliably sexed based on external morphology, whereas deutonymphs (Condé's stages B for females and C for males) can be easily separated, as can the adults. These three instars can be readily recognized by a number of morphological characters, and are morphometrically quite distinct.

The life history of prokoeneniids is also apparently quite simple and consists of four active instars: protonymph (Rucker's "First Known Stage"), deutonymph (Rucker's "Second Stage"), tritonymph (Rueker's "Last Stage before the adult") and adult. There is no evidence whatsoever for a "prelarva" as proposed by Van der Hammen, nor is there any support for the intermediate instar (between Rucker's First and Second Stages) that the same author proposed. Examination of the "size-comparable" figures in Rucker's paper, drawn at the same scale and magnification, show only that the full, ventral views of the abdomen are comparable. Based on measurements obtained from a printed copy of 39mm, 55mm, 65mm and 71mm in total length; we obtain growth factors of 1.41, 1.18 and 1.09 between proto- and deuto-, between deuto- and tritonymph, and between tritonymph and adult, which is a rather high growth factor for the first molt.

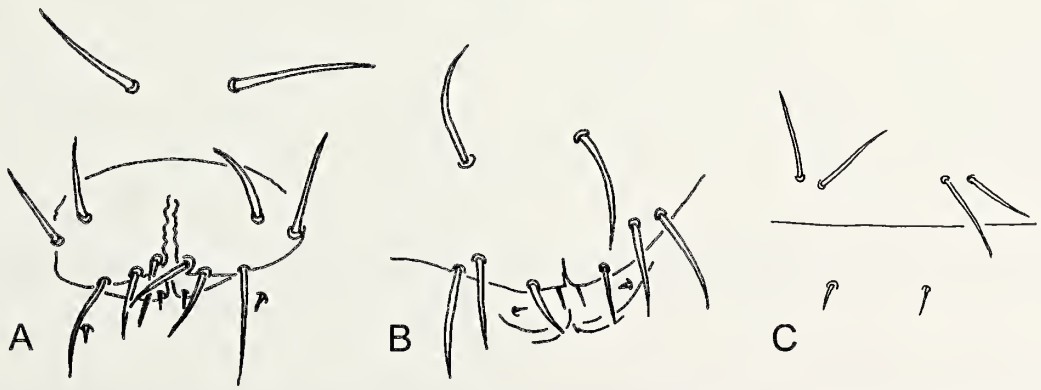

Figure 2.—Sex determination in juvenile *Eukoenenia chilanga*, from Tlanepantla, Estado de México. Genital region.- A. Male deutonymph, B. Female deutonymph, C. protonymph.

Table 3.—Results from the principal component analysis for the palpigrades from Tlalnepantla, Estado de México. Eigenvalues on the left, and eigenvectors on the right-hand columns, respectively. Bold type indicates the characters that account for most of the variation in the PCA analysis.

Eigenvalues					Eigenvectores		
Eigenvalue (Total = 27.00000)	Percentage	Cumulative	#	Characters	C1	C2	C3
23.7882	**88.1045**	**88.1045**	**1**	**number of ventral setae on propeltidium**	**0.9742**	0.0209	0.0086
0.59833	**2.2160**	**90.3206**	**2**	**number of lateral organs**	**0.9850**	0.0283	0.0190
0.42266	1.5654	91.8860	3	**number of ventral setae on sternite X**	**0.9094**	−0.1780	0.2370
0.39163	1.4505	93.3364	4	**number of ventral setae on sternite XI**	**0.9070**	−0.2016	0.2288
0.27035	1.0013	94.3377	5	**number of ventral setae on sternite XII**	**0.9586**	0.07120	0.0909
0.22416	0.8302	95.1679	6	**length of basal segment of chelicera**	**0.9883**	0.0050	0.0312
0.20227	0.7492	95.9171	7	**length of medial seta on basal segment of chelicera**	**0.9346**	−0.1030	−0.1304
0.18575	0.6880	96.6051	8	**length of movable finger of chelicera**	**0.9242**	0.0813	−0.0755
0.15756	0.5836	97.1886	9	**length of pedipalp trochanter**	**0.9548**	0.1138	−0.0059
0.13787	0.5106	97.6993	10	**length of pedipalp tibia**	**0.9684**	0.1740	0.0297
0.11565	0.4283	98.1276	11	**length of trochanter I**	**0.9570**	0.0270	0.1303
0.08795	0.3257	98.4533	12	length of latero-dorsal seta on trochanter I	0.8205	−0.1047	0.3072
0.08052	0.2982	98.7516	13	**length of tibia I**	**0.9506**	0.1695	0.0031
0.06326	0.2343	98.9858	14	**length of the solenidium on basitarsus 3 of leg I**	**0.9413**	−0.0769	−0.1336
0.05763	0.2135	99.1993	15	length of dorso-proximal seta on basitarsus 7 of leg II	0.8999	−0.1669	−0.1435
0.04512	0.1671	99.3664	16	**length of basistarsus 2 on leg II**	**0.9487**	0.1557	−0.1284
0.03633	0.1346	99.5010	17	**length of dorsolateral seta on trochanter III**	**0.9074**	−0.0954	−0.2637
0.03236	0.1198	99.6208	18	**length of tibia III**	**0.9129**	0.2440	0.0027
0.03012	0.1115	99.7324	19	**length of ventro-lateral seta on tibia III**	0.8904	−0.3014	−0.0167
0.02481	0.0919	99.8243	20	**length of basitarsus 2 on leg III**	**0.9667**	0.1127	0.0780
0.01551	0.0575	99.8817	21	length of solenidium on basitarsus 3 of leg III	0.8938	−0.2250	−0.1518
0.01245	0.0461	99.9278	22	**length of patella IV**	**0.9798**	0.1323	0.0255
0.00717	0.0266	99.9544	23	**length of tibia IV**	**0.9823**	0.1160	0.0137
0.00566	0.0210	99.9753	24	**length of ventrodistal seta on tibia IV**	**0.9005**	−0.2561	−0.0633
0.00359	0.0133	99.9886	25	**length of basitarsus 1 on leg IV**	**0.9691**	0.0619	0.0400
0.00181	0.0067	99.9954	26	**length of insertion of solenidium on basitarsus 1 on leg IV**	**0.9400**	0.1776	−0.0920
0.00125	0.0046	> 100%	27	**length of the solenidium on basitarsus 1 of leg IV**	**0.9574**	−0.0777	−0.0255

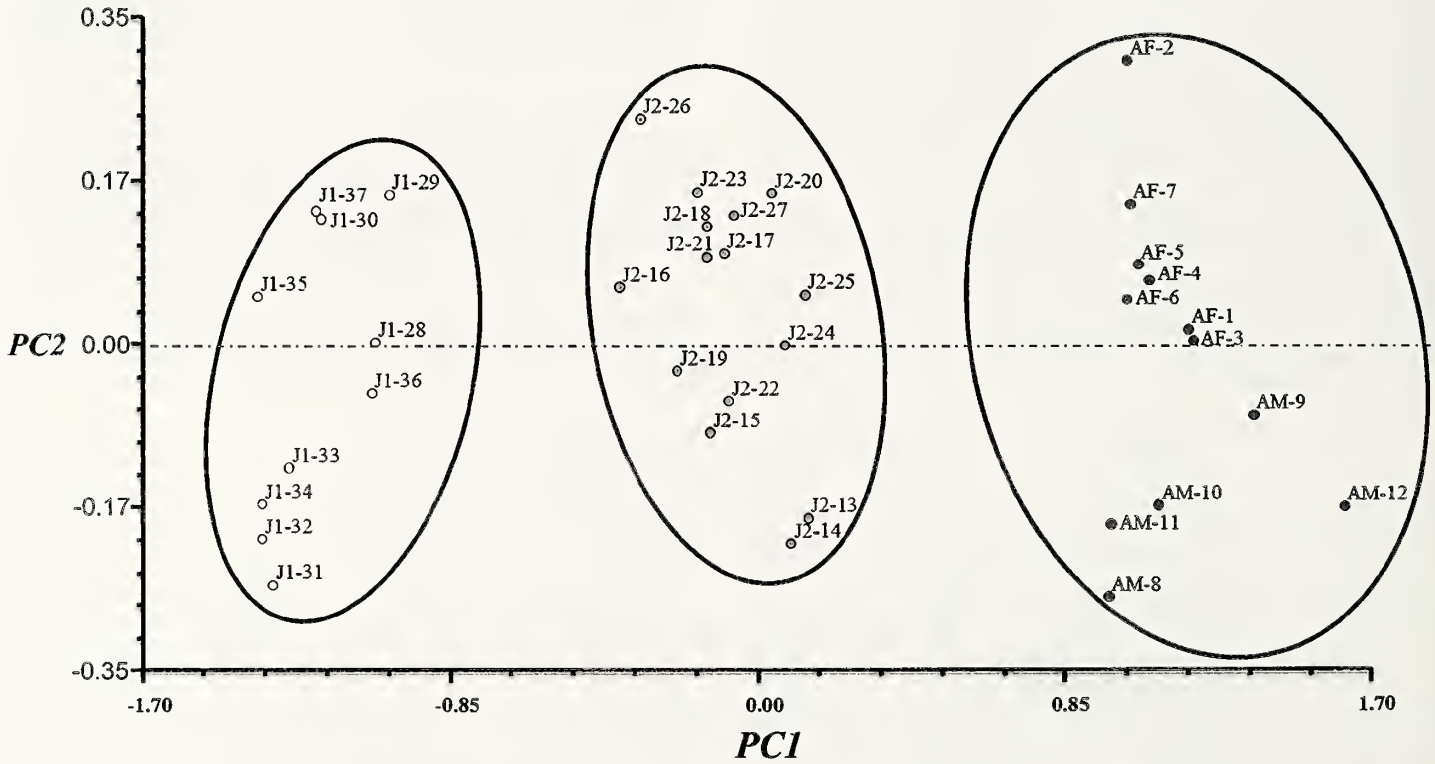

Figure 3.—Ontogeny of *Eukoenenia chilanga*. Graphic representation of the results of the principal components analysis showing the separation of three age classes (from left to right protonymph, deutonymph and adult) along the ordinate, and the separations by sexes along the abscissa (males below 0, females above 0; AF = adult female, AM = adult male).

Table 4.—Calculation of growth factors (GF) between instars for six different structures (lengths in microns) in female and male palpigrades (XP =average in protonymphs, XD = average in deutonymphs, XA = average in adults).

	Characters	XP	GF	XD	GF	XA
♀	Basal cheliceral segment	92.48	1.193	108.96	1.178	130.05
	Tibia IV	63.68	1.218	77.28	1.213	94.17
	Patela IV	68.16	1.172	82.08	1.204	96.22
	Basitarsus IV	50.56	1.168	64.96	1.284	75.88
	Pedipalp tibia	65.28	1.185	80.8	1.237	95.77
	Basitarsus 2 on leg III	41.92	1.206	53.6	1.278	64.68
♂	Basal cheliceral segment	131.52	1.208	108.8	1.227	88.64
	Tibia IV	93.44	1.242	75.2	1.270	59.2
	Patela IV	96.64	1.203	80.32	1.267	63.36
	Basitarsus IV	77.12	1.181	65.28	1.333	48.96
	Pedipalp tibia	95.68	1.245	76.8	1.311	58.56
	Basitarsus 2 on leg III	63.68	1.213	52.48	1.378	38.08

However, if we measure the width of the genital sternite we obtain 1.5mm, 1.7mm, 1.9mm and 2.2mm for corresponding growth factors of 1.13, 1.12 and 1.16—closer to those we observed in *E. chilanga*. It is known that the length of the abdomen varies with the feeding condition of the animals, and this probably explains the "important gap between the sizes of Rucker's first and second known instars", which prompted Van der Hammen to propose the existence of an intermediate, additional instar in the life history of prokoeneniids.

The sex ratio in many palpigrades is highly skewed toward females (Condé 1984), to the extent that parthenogenesis has been suggested as a possible explanation for the lack, or near lack, of males in some populations. However, in *E. chilanga* the sex ratio observed does not deviate significantly from 50:50.

It is necessary to emphasize that the terminology used by Bruno Condé for the life stages or instars (A1, A2, B and C), are atypical in the terminology widely accepted for the Class Arachnida, so the authors request that the arachnological community homologize those terms and in the future refer to palpigrade life stages as protonymph, deutonymph, tritonymph (when present, as in the case of *Prokoenenia*) and adult.

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Appendix 1.—Data base matrix of the principal component analysis.

	Characters	AF-1	AF-2	AF-3	AF-4	AF-5	AF-6	AF-7
1	Number of ventral setae on propeltidium	5	5	6	4	5	5	5
2	Number of lateral organs	3	3	3	3	3	3	3
3	Number of ventral setae on sternite X	10	10	10	10	10	10	10
4	Number of ventral setae on sternite XI	10	10	10	10	10	10	10
5	Number of ventral setae on sternite XII	10	10	10	10	10	10	10
6	Length of basal segment of chelicera	132.8	128	136	132.8	128	124.8	128
7	Length of medial seta on basal segment of chelicera	62.4	57.6	64	62.4	57.6	59.2	60.8
8	Length of movable finger of chelicera	81.6	80	78.4	83.2	80	75.2	81.6
9	Length of pedipalp trochanter	88	96	94.4	91.2	91.2	92.8	91.2
10	Length of pedipalp tibia	96	96	96	96	96	92.8	97.6
11	Length of trochanter I	84.8	88	83.2	84.8	81.6	84.8	80
12	Length of latero-dorsal seta on trochanter I	83.2	64	81.6	80	80	73.6	72
13	Length of tibia I	102.4	104	100.8	99.2	99.2	100.8	104
14	Length of the solenidium on basitarsus 3 of leg I	35.2	35.2	33.6	33.6	33.6	35.2	35.2
15	Length of dorso-proximal seta on basitarsus 7 of leg II	65.6	70.4	72	68.8	68.8	68.8	65.6
16	Length of basistarsus 2 on leg II	59.2	57.6	60.8	59.2	56	56	57.6
17	Length of dorsolateral seta on trochanter III	88	91.2	96	96	91.2	88	91.2
18	Length of tibia III	57.6	56	51.2	54.4	56	52.8	56
19	Length of ventro-lateral seta on tibia III	38.4	27.2	33.6	33.6	32	32	33.6
20	Length of basitarsus 2 on leg III	65.6	62.4	68.8	62.4	65.6	64	64
21	Length of solenidium on basitarsus 3 of leg III	27.2	24	25.6	24	25.6	25.6	25.6
22	Length of patella IV	99.2	99.2	92.8	94.4	96	96	96
23	Length of tibia IV	96	96	94.4	91.2	91.2	94.4	96
24	Length of ventrodistal seta on tibia IV	32	28.8	32	32	32	32	28.8
25	Length of basitarsus 1 on leg IV	75.2	76.8	73.6	76.8	75.2	78.4	75.2
26	Length of insertion of solenidium on basitarsus 1 on leg IV	38.4	40	40	40	40	38.4	36.8
27	Length of the solenidium on basitarsus 1 of leg IV	41.6	41.6	43.2	40	41.6	43.2	41.6

Appendix 1.—Extended.

AM-8	AM-9	AM-10	AM-11	AM-12	J2-13	J2-14	J2-15	J2-16	J2-17	J2-18	J2-19	J2-20	J2-21	J2-22
5	5	5	5	5	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	2	2	2	2	2	2	2	2	2	2
13	14	16	15	17	8	8	8	8	8	8	8	8	8	8
16	16	15	14	16	8	8	8	8	8	8	8	8	8	8
10	10	10	10	10	8	8	8	8	8	8	8	8	8	8
128	132.8	129.6	129.6	137.6	115.2	112	110.4	107.2	110.4	107.2	100.8	108.8	107.2	105.6
56	56	60.8	62.4	62.4	52.8	56	51.2	46.4	51.2	52.8	46.4	49.6	48	46.4
80	81.6	80	76.8	70.4	65.6	70.4	67.2	67.2	68.8	70.4	65.6	67.2	65.6	68.8
83.2	89.6	91.2	86.4	102.4	78.4	80	78.4	72	76.8	75.2	76.8	81.6	78.4	78.4
92.8	99.2	96	91.2	99.2	76.8	75.2	75.2	75.2	76.8	80	78.4	84.8	80	78.4
83.2	88	86.4	80	94.4	72	76.8	78.4	72	75.2	67.2	72	67.2	70.4	72
75.2	83.2	73.6	76.8	84.8	76.8	70.4	67.2	67.2	68.8	68.8	62.4	68.8	62.4	60.8
96	105.6	102.4	96	102.4	86.4	75.2	76.8	81.6	83.2	86.4	83.2	88	88	86.4
35.2	36.8	33.6	33.6	38.4	28.8	32	28.8	25.6	27.2	28.8	27.2	32	30.4	32
68.8	72	62.4	65.6	72	60.8	64	59.2	52.8	57.6	56	56	59.2	60.8	64
51.2	60.8	56	51.2	60.8	46.4	46.4	46.4	43.2	48	51.2	46.4	52.8	43.2	49.6
89.6	96	88	91.2	97.6	86.4	86.4	81.6	73.6	80	80	78.4	84.8	73.6	83.2
48	56	51.2	51.2	57.6	46.4	43.2	41.6	44.8	48	41.6	41.6	48	48	41.6
33.6	35.2	36.8	33.6	38.4	32	30.4	28.8	27.2	22.4	24	25.6	24	24	28.8
60.8	68.8	62.4	57.6	68.8	51.2	54.4	52.8	51.2	54.4	52.8	49.6	52.8	52.8	54.4
27.2	27.2	25.6	25.6	27.2	25.6	24	20.8	19.2	22.4	20.8	24	24	24	20.8
89.6	102.4	94.4	92.8	104	84.8	80	80	76.8	83.2	81.6	78.4	84.8	83.2	78.4
88	96	94.4	88	100.8	76.8	73.6	75.2	72	78.4	76.8	76.8	78.4	75.2	73.6
32	32	32	32	33.6	28.8	27.2	27.2	25.6	27.2	24	27.2	24	24	28.8
73.6	83.2	73.6	72	83.2	72	64	62.4	59.2	62.4	64	64	67.2	65.6	64
36.8	36.8	33.6	40	41.6	33.6	33.6	30.4	30.4	32	33.6	32	35.2	32	32
41.6	38.4	44.8	40	46.4	36.8	38.4	35.2	32	33.6	35.2	35.2	33.6	35.2	33.6

Appendix 1.—Extended.

J2-23	J2-24	J2-25	J2-26	J2-27	J1-28	J1-29	J1-30	J1-31	J1-32	J1-33	J1-34	J1-35	J1-36	J1-37
3	3	3	3	3	2	1	1	1	1	1	1	1	1	1
2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
8	8	8	8	8	4	4	5	4	4	4	4	4	4	4
8	8	8	8	8	4	4	4	4	4	4	4	4	4	4
8	8	8	8	8	4	4	4	4	4	4	4	4	4	4
110.4	110.4	112	108.8	107.2	94.4	92.8	94.4	89.6	89.6	88	84.8	91.2	91.2	89.6
49.6	49.6	49.6	44.8	49.6	46.4	46.4	40	46.4	43.2	43.2	41.6	40	43.2	41.6
68.8	72	64	67.2	60.8	59.2	60.8	60.8	56	56	57.6	57.6	59.2	59.2	57.6
83.2	83.2	80	80	80	62.4	67.2	67.2	67.2	67.2	57.6	56	62.4	67.2	64
83.2	83.2	83.2	80	81.6	68.8	68.8	57.6	54.4	54.4	62.4	54.4	64	67.2	67.2
76.8	76.8	80	72	73.6	62.4	64	60.8	59.2	59.2	59.2	54.4	60.8	60.8	62.4
70.4	65.6	92.8	65.6	64	59.2	60.8	59.2	56	59.2	62.4	57.6	56	54.4	49.6
83.2	89.6	89.6	80	84.8	75.2	72	67.2	52.8	64	75.2	60.8	67.2	72	72
27.2	32	30.4	25.6	30.4	27.2	22.4	24	24	24	22.4	25.6	24	27.2	24
56	57.6	62.4	56	56	49.6	51.2	48	60.8	56	52.8	51.2	49.6	49.6	51.2
46.4	46.4	46.4	43.2	49.6	41.6	41.6	40	35.2	33.6	38.4	38.4	36.8	40	43.2
68.8	88	75.2	76.8	83.2	72	76.8	72	70.4	68.8	78.4	70.4	65.6	72	72
46.4	41.6	46.4	46.4	48	41.6	43.2	43.2	36.8	35.2	33.6	38.4	35.2	38.4	40
24	25.6	25.6	22.4	20.8	24	19.2	19.2	24	17.6	17.6	24	19.2	20.8	20.8
56	54.4	57.6	52.8	51.2	43.2	46.4	43.2	36.8	35.2	38.4	43.2	38.4	36.8	38.4
20.8	24	20.8	19.2	22.4	19.2	17.6	19.2	19.2	20.8	19.2	19.2	17.6	20.8	19.2
81.6	83.2	83.2	80	83.2	65.6	70.4	72	64	62.4	62.4	59.2	65.6	68.8	67.2
73.6	80	81.6	76.8	80	64	64	67.2	57.6	59.2	57.6	57.6	62.4	64	60.8
25.6	27.2	27.2	22.4	28.8	24	24	20.8	22.4	24	24	22.4	20.8	27.2	19.2
62.4	67.2	73.6	62.4	65.6	51.2	54.4	46.4	46.4	48	46.4	48	48	56	52.8
30.4	32	33.6	32	35.2	25.6	30.4	27.2	24	22.4	24	24	25.6	28.8	28.8
33.6	36.8	36.8	32	33.6	25.6	28.8	25.6	25.6	27.2	30.4	28.8	27.2	30.4	28.8

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SHORT COMMUNICATION

Third pair of legs is a key feature for eliciting female receptivity in the road tarantula spider *Eupalaestrus weijenberghi* (Araneae: Theraphosidae)

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Abstract. Using the road tarantula *Eupalaestrus weijenberghi* (Thorell 1894), we tested the importance of the third pair of legs in male courtship behavior. Our results showed that the third pair of legs is essential for males to elicit female sexual receptivity. Males with their second pair of legs immobilized elicited reeeptive responses from females, but males with the third legs immobilized did not. The potential role of the third pair of legs in the generation and/or transmission of seismic signals via the substrate is discussed.

Keywords: Courtship, leg vibration, seismic communication

In theraposids, males usually perform body vibration, palpal drumming, and leg tapping during courtship (Costa & Pérez-Miles 2002; Pérez-Miles et al. 2005, 2007; Almeida-Silva et al. 2008; Ferretti & Ferrero 2008). These body vibrations constitute an important communication channel, mainly for burrowing theraphosids (Quirici & Costa 2005). Although Costa & Pérez-Miles (2002) highlighted the use of leg III in body vibrations, the importance of the vibrations generated and/or transmitted by leg III to the substrate and their implications in female receptivity remain obscure. Here we present experimental evidence of the role of the leg III pair in female reeeptivity in the theraposid species, *Enpalaestrns weijenberghi* (Thorell 1894).

Enpalaestrus weijenberghi is a medium-sized tarantula that inhabits burrows in meadows of the Pampean biogeographic province (see Pérez-Miles et al. 2005). The reproductive season of this species is between February and April (autumn in the Southern Hemisphere). Males search for females using chemical cues and live only two months as adults, while females are sedentary and live up to ten years as adults (Costa & Pérez-Miles 2002; Pérez-Miles et al. 2005). As in other mygalomorphs, females continue molting through their adult stage. During the molt, the lining of the seminal receptacles is shed and the females become "virgin" again, needing new sperm after each molt (Foelix 2011). Therefore, these new virgin, sexually receptive females call males by tapping the substrate with their forelegs. Males can perform multiple matings, whereas females are monandric in each reproductive season, which occurs in alternate years and is associated with the year that they molt again (Pérez-Miles et al. 2007).

For this study, we collected male *E. weijenberghi* from Southern Uruguay, Canelones Salinas Norte (34°44′56″S, 55°52′15″W) and from neighboring areas in February and March 2011. We used female *E. weijenberghi* from our laboratory populations, which were maintained as in Costa & Pérez-Miles (2002) for at least one year before the experiments; they also originated from Southern Uruguay. Only those females that had molted within six months of the experiment were used in the experimental trials in order to guarantee their need for sperm and, therefore, their potential receptivity. Females were individually maintained in containers (50 cm length, 15 cm width, and 20 cm height) containing a layer of soil. We constructed artificial burrows, similar to those found in the field (see Pérez-Miles et al. 2005, 2007), against the glass walls of the containers, to facilitate observation. We maintained the males in cylindrical glass containers of 7.5 cm diameter with soil for at least one week before the trials. All individuals were feed ad libitum with *Blaptica dubia* (Serveille 1839) (Blataria: Blaberidae). We designed three experimental treatments: in one of them (pair-three-tied group), the third pair of the male legs was tied between them at the joint of the patella and femur, above the carapace, using cotton threads (Fig. 1). In a second treatment, we tied pair two in the same way (pair-two-tied group); in a third treatment (control group), males remained with their legs free but were manipulated to simulate the ligature. Five minutes prior to the experimental trials, we manipulated or tied the legs of the males. After this period, males were slowly introduced into the females' container. Males and females experienced all the treatments in a random sequence over consecutive days; however, couples were never repeated.

In each experimental trial, we recorded the number of male body vibrations, female calling (tapping the first and second pair of legs against the substrate), and female rejection of males (piston behavior, attacks or abrupt emergence). Piston behavior consisted in forward and backward movements of the female, usually in the burrow. For detailed descriptions of courtship behaviors see Costa and Pérez-Miles (2002) and Quirici & Costa (2005). We finished the experimental trials when the female attacked the male, when the female accepted clasping (male clasped female chelicerae with his tibial apophyses before mating), or 30 min after the male was introduced. We interrupted the experimental trials after clasping, avoiding copulation (to retain female virgins and sexual receptivity). We performed all trials in three consecutive days (9–11 March 2011). During the experimental trials, the room temperature varied between 26 and 28°C, with a mean of 26.9° C (± 0.6 SD). Statistical analyses were carried out using the Past package (Paleontological Statistics version 2.05, Hammer et al. 2010). Bonferroni-corrected critical values ($\alpha = 0.0167$) were used in McNemar and Wilcoxon test results for multiple comparisons.

We observed that males performed courtship behavior in all 21 trials of the control group, in 19 of the pair-two-tied group, and in 19 of the pair-three-tied group. Females called the males in 16 trials of the control group, in 16 of the pair-two-tied group, and in 3 of the pair-three-tied group. Using the Cochran Q-test for repeated measures, we compared female calling among the three groups and found that it varied significantly with male treatment ($\chi^2_Q = 14.700$, $P = 0.001$). In the pair-wise comparisons for repeated measures (McNemar test), the pair-three-tied group differed from the control group ($P = 0.002$) and from the pair-two-tied group ($P = 0.007$),

Figure 1.—Male *Eupalaestrus weijenbergli* with the third pair of legs tied with a cotton thread.

whereas the control and pair-two-tied groups showed no significant differences between them ($P = 0.72$). We did not find significant differences in the latency of female calling between pair-two-tied and control groups using the non-parametric Wilcoxon test for the pairwise comparisons ($Z = 1.57$, $P = 0.12$). We did not compare the latency of the female call in pair-three-tied group because only three females responded. We also tested whether the female's response to the male's treatment changed over time, but found no statistical differences in the latency of calling behavior over the days of the experiment (Kruskal-Wallis, $H = 0.26$, $P = 0.88$). Four females rejected males (one attack and three piston behaviors) in the leg-three-tied group, while we did not observe any rejection in the other groups.

The latency of male courtship was 5.04 ± 4.46 min in the control group, 5.82 ± 5.20 min in the leg-two-tied group and 9.43 ± 9.67min in the leg-third-tied group. We did not find significant differences using the Friedman test for repeated measures ($\chi^2 = 1.08$, $P = 0.59$). Male body vibrations occurred at 1.90 ± 1.22 bouts per min in the control group, 2.00 ± 2.20 per min in the leg two-tied-group, and 1.04 ± 0.93 per min in the leg-three-tied group. We found significant differences in rates of body vibration between the leg-three-tied group and the control group using the Wilcoxon test for pairwise comparisons ($Z = 2.43$, $P = 0.015$). However, we did not find significant differences between the leg-two-tied group and the leg-three-tied group ($Z = 1.82$, $P = 0.068$) or the control group ($Z = 0.40$, $P = 0.68$).

We conclude that the third leg is essential for female receptivity. The experimental tying of legs II or legs III did not inhibit male courtship behavior, but affected female sexual response. The absence of differences in the latency of male courtship showed that males will start courtship behavior, regardless of the treatment, when they are in contact with female silk threads. Furthermore, males with the third legs tied did not differ statistically in body vibration regardless of the leg-two-tied groups, suggesting that which legs have been tied does not appear to have an important effect in this stereotyped behavior, and also because females called to males with the second legs tied. In addition, we did not find significant differences in the frequencies of vibratory bouts between the control and the leg-two-tied group. However, when males had their third legs tied, their body vibrations on average were less frequent than with legs two tied and also statistically different from the control groups. These results suggest the importance of the third free leg for increasing body vibrations and also for a complete female response. When third legs were tied, spasmodic movements of the third pair were observed. Most likely, transmission through the remaining free legs was not adequate to elicit female sexual response. We did not observe such spasmodic movements when the second pair was tied. Legs of the third pair, when free, are involved in the lateral equilibrium of the body, and they can be firmly placed on the substrate on both sides of the body. Legs I and II are placed in front of the body, and legs IV are placed in the back of the body, and these legs are responsible for the transmission of body vibrations from the front to the back and from the back to the front of the male's body. Therefore, legs III are in the best position and are the best candidates for transmitting vibrations from the top to the bottom of the body (F.G. Costa pers. observ.). This relationship allows the spiders to transfer the mechanical energy produced by the leg muscle contractions to the substrate and to vibrate their bodies. A comparable mechanism was proposed for the sparassid *Heteropoda venatoria* (Linnaeus 1767) by Rovner (1980) and by Rovner & Barth (1981) for the ctenid *Cupiennius salei* (Keyserling 1877).

We could not attribute the differences in female behavior to differences in the frequency of male sexual display because we did not find any significant differences in the male sexual behavior between the two groups with tied legs. Consequently, the third legs appear to be key features for eliciting a positive sexual response from the female. As far as we know, this is the first experimental evidence of a mechanism underlying this widespread courtship behavior in tarantulas and other mygalomorph spiders.

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SHORT COMMUNICATION

Opening and closing of burrows by the Namibian spider *Ariadna* sp. (Araneae: Segestriidae) in a year of heavy rainfall

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Abstract. The *Ariadna* spiders (Araneae: Segestriidae) inhabiting the gravel plains of the Central Namib Desert construct individual burrows with a circular entrance surrounded by a ring of small pebbles; sometimes they close their burrows by a small stone. In the lichen fields, about 20 km east of Walvis Bay (Namibia), there is a consistent population of *Ariadna* spiders that can also use pieces of lichen both in the ring composition and as the plug when the burrow is closed. We sampled and monitored 175 burrows repeatedly between December 1999 and August 2000. In Mareh 2000, an exceptionally high rainfall occurred in the Namib, leading to flooding even in our fieldwork station. We tested whether and to what extent an event of this magnitude could affect burrow closing. We found the rain event increased burrow closure by large, but not small or medium-sized, *Ariadna* sp. We suggest that the flooding event acted as an ecological resource pulse for these spiders.

Keywords: Flooding, lichen-stone fields, Namib Desert, spider population, survival strategies

Spiders of the genus *Ariadna* (Araneae: Segestriidae) inhabiting the gravel plains of the Central Namib Desert dig individual, silk-lined burrows and surround their circular entrances by rings of small quartz stones, suitably selected during the digging activity (Costa et al. 1993, 1995; Henschel 1995). Spiderlings, whose body size is just over a millimeter, simply place a series of sand grains around the mouth of their burrows (Costa et al. 1993, 1995). The features of these rings vary according to the populations and their habitat; in some areas of the Namib Desert, where wide plains rich in lichens occur, pieces of lichen can also be ineluded in the rings.

A spider lurking at the bottom of its burrow keeps the entrance open in order to drag prey inside; the ring items, by transmitting vibrations produced by walking prey, serve as foraging tools allowing spiders to expand their sensory range (Henschel 1995). We observed that these spiders sometime close their burrows, plugging the entrance with a stone or a piece of lichen (Costa et al. 2000), probably as an anti-predatory strategy after consuming prey. The burrows are later reopened when they hunt again.

The Namib Desert is a hyperarid desert stretching from South Africa, through Namibia, and into Angola (Goudie 2002). It is characterized by diurnal high temperature and scanty, irregular rainfall; in the Namib in particular, rainfall is not a predictable factor but does increase from the coast inland (Viles 2005). Mean annual rainfall ranges from 18 mm at Swakopmund, on the coast, to 50 mm at Ganab, located over 100 km inland (Lancaster et al. 1984). In contrast to rainfall, fog is a more predictable factor (Shanyengana et al. 2002). It is heavy along the coast, decreasing inland, and represents an important alternative source of moisture that is crucial for the survival of plants and animals (Costa 1995). Due to the influence of the cold seawater and the frequent occurrence of fog, temperatures are cool and show little diurnal variation along the coast, while there is a nearly continuous high relative humidity. Wind is also a very important component of the central Namib climate, reaching up to 80 km/h and frequently causing sandstorms (Seely 1987). Against this background of low rainfall and coastal fog, every 16–20 years rare rain pulses do occur in the Namib Desert (Hachfeld 2000). In the rainy season of 1999/2000, exceptionally high rainfall occurred in the Central Namib, with 78 mm of precipitation on 24 and 25 March 2000 (Hachfeld 2000).

Our study site, about 20 km east of Walvis Bay, Namibia, along the C14 highway in the Namib Naukluft Park (23°00′32.7″S, 14°43′38.0″E; altitude 46 m), is level and rich in lichens, part of 'Lichen field I' of the Central Namib as defined by Schieferstein & Loris (1992). The ground is made up of fine gravel consisting mainly of quartz stones of many shapes and sizes (Costa et al. 2000). As part of our long-term research on the behavioral adaptations of Namib Desert arthropods, from December 1999 to August 2000 we carried out an eco-ethological survey on a population of an undescribed *Ariadna* species, whose individuals may include quartz stones and/or lichen pieces around the openings of their burrows, sometimes forming a turret (Costa et al. 2000). During the study period, the mean temperature was 18.1°C (min 16.1°C in December 1999, max 20.0°C in January 2000), while the mean humidity was 71.7% (min 59.2% in June 2000, max 80% in February 2000).

The exceptional rain of March 2000 stimulated us to investigate whether and to what extent an event of this magnitude could affect the behavior of burrow closing by *Ariadna* spiders.

We divided our 50 × 80 m fieldwork area into 160 5×5-m squares using a grid system composed of nylon string and wooden stakes. From 19 to 27 December 1999, we located and marked 175 spider burrows with numbered flags. We recorded the burrow entrance diameter (measured with a Vernier caliper, measurement error = 0.05 mm), the status of the entrance (open or closed), and counted stones and lichen pieces placed around the lip of the burrow entrance by the spiders. Then in five different months (15 January, 9 February, 19 April, 15 May and 9 August), we ascertained whether each burrow was open or closed (data for all 175 burrows were collected in one day for each sampling). Since these spiders tend to remain in the same burrows and widen the walls of them as they grow (Henschel 1995), it is possible to assume that the diameter of the burrow entrance is age-dependent as also occurs with other spiders (Carrel 2003). Assuming that the measures of burrow entrance diameter correlate to spider age, we sorted the 175 diameters into three size classes: 'small' (spiderlings, diameter less than 3.0 mm, $n = 62$), 'medium' (sub-adults, diameter greater than or equal to 3.0 mm and less than 4.5 mm, $n = 46$), and 'large' (adults, diameter greater than or equal to 4.5 mm, $n = 67$). Since the long-lived Namibian *Ariadna* spiders widen their burrows by less than 1 mm per year (Henschel 1995), we have considered the variation of the diameter of the sampled burrows to be negligible during our study period (nine months), also taking into account that such variation, from a monthly sampling to the next one, was

comparable to the measurement error, and so we do not consider those data here.

We evaluated whether the ring composition (number of stones or pieces of lichen) correlated with the burrow diameter. Then, to test the influence of the rainfall on opening and closing of burrows, we a) assigned the value 0 to each open burrow and the value 1 to each closed burrow, b) grouped the monthly data into three time variables [time 1, pre-rain (data from December 1999, January and February 2000), time 2, immediately post-rain (data from April) and time 3, months-after rain (data from May and August)] and reduced the influence of the differenees in the number of months in the three time periods by using mean values for each individual; c) used a mixed design, repeated measures ANOVA, with one between-group effect (size-class) for time 1, time 2 and time 3 and one within-subject effect (time period) on the opening/closing behavior data from time 1, time 2 and time 3. To compare the status of burrows between the first (December 1999) and the last (August 2000) samplings, we used McNemar's test for paired-sample nominal scale data. Statistical analyses were conducted using SPSS for Windows (version 18.0).

We found that the number of pieces of lichen and diameter of the entrance burrow were positively correlated ($r = 0.60$, $P < 0.001$); the regression line ($y = -0.71 + 0.89x$) had a significant slope ($P \ll 0.001$).

Repeated measures ANOVA showed a highly significant difference in closed burrows between the three size-classes ($F = 12.16$; $df = 2, 172$; $P \ll 0.001$). Moreover, the interaction of time and size class was also significant ($F = 30.45$; $df = 3.86, 331.80$; $P \ll 0.001$). Post-hoc Bonferroni comparisons showed that the number of closed burrows was significantly different between small and medium burrows ($P < 0.02$) and small and large burrows ($P \ll 0.001$), while medium and large burrows did not differ. Rainfall significantly increased the closing behavior overall ($F = 15.28$; $df = 1.93, 331.80$; $P \ll 0.001$). Post-hoc Bonferroni comparisons revealed significant differences between time 1 and time 2 ($P \ll 0.001$) and between time 2 and time 3 ($P \ll 0.001$), but no significant differences between time 1 and time 3 (Table 1). Finally, the McNemar's test showed no significant difference in number of closed burrows between the first and the last sampling ($n = 66$ and $n = 75$ respectively) ($\chi^2 = 0.93$).

Our results on closing/opening frequency of *Ariadna* burrows over time (Fig. 1, Table 2) suggest that 1) the heavy rainfall of March 2000 influenced the closing/opening of burrows in some spiders; 2) once the rain event ended, adults tended to keep their large burrows closed for weeks, whereas smaller spiders opened their burrows soon thereafter, if they closed them at all; 3) the proportion of open small burrows increased over time (rising from 0.2 to 0.5); 4) the proportion of closed medium size burrows (0.5) did not change significantly over time.

Exceptional rain events can change the landscape and endanger survival in desert environments, compelling arid-adapted organisms to resort to alternative strategies (Cloudsley-Thompson 1983). Indeed, adults of *Ariadna* spiders in this work appeared to cope with the flooding by plugging their burrows long-term with a stone or a lichen piece. It remains to be determined whether small and medium size spiders exhibit the same behavior on a short-term basis, such as during the downpour itself and a few days thereafter.

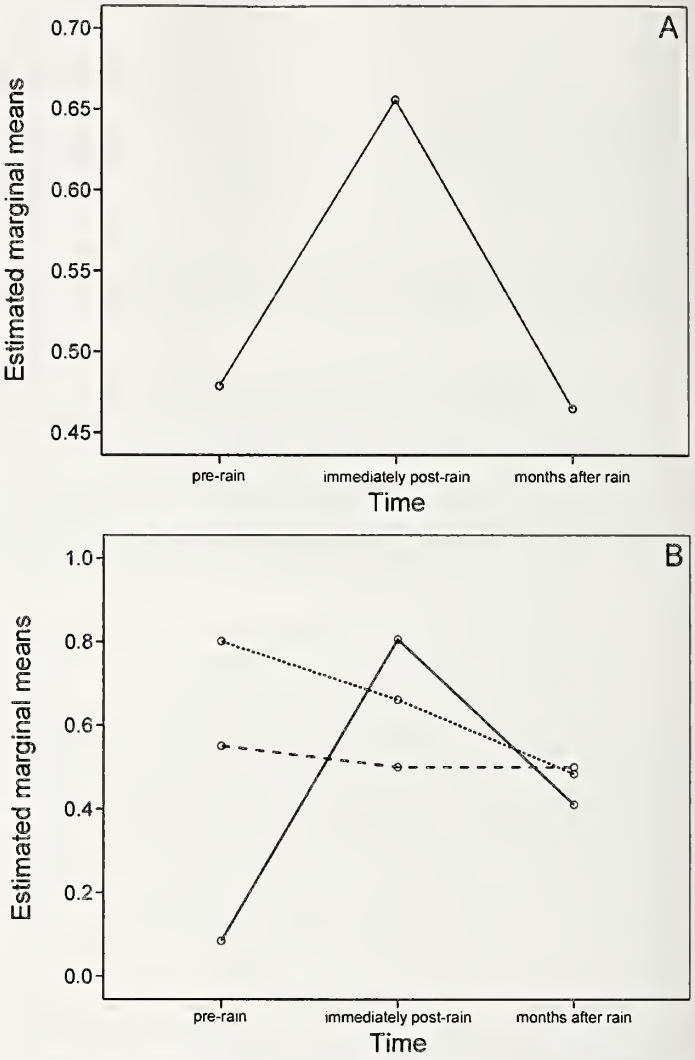

Figure 1.—Plot of estimated marginal means of proportions of closed burrows of *Ariadna* sp. in the three different time periods with respect to rainfall of March 2000: A) all 175 burrows, B) each size class. Values in the graphs refer to the mean values of closed burrows as specified in Table 2. Symbols for graph B: dotted line = small burrows, dashed line = medium burrows, continuous line = large burrows.

It is well known that many terrestrial arthropods are able to withstand prolonged submersion in water (Cloudsley-Thompson 1958). Interestingly, a closely related species, *Ariadna bicolor* (Hentz, 1842), was shown to survive long periods of experimental submersion,

Table 1.—Post-hoc Bonferroni comparisons for rainfall effect based on estimated marginal means of all *Ariadna* closed burrows.

(I) Time	(J) Time	Mean difference (I–J)	SE	*P*
pre-rain ($m = 0.461$)	immediately post-rain	−0.177*	0.042	0.000
	months-after rain	0.014	0.037	1.000
immediately post-rain ($m = 0.674$)	pre-rain	0.177*	0.042	0.000
	months-after rain	0.191*	0.037	0.000
months-after rain ($m = 0.460$)	pre-rain	−0.014	0.037	1.000
	immediately post-rain	−0.191*	0.037	0.000

Table 2.—Statistical parameters of proportions of closed burrows of the *Ariadna* sp. inhabiting a Namibian gravel-lichen plain according to mm-size class of the entrance diameter and the different time periods (pre-, immediately post- and months-after rain).

Group	Time	Mean	Std. Error
small	pre-rain	0.801	0.038
	immediately post-rain	0.661	0.058
	months-after rain	0.484	0.048
medium	pre-rain	0.551	0.044
	immediately post-rain	0.5	0.067
	months-after rain	0.5	0.056
large	pre-rain	0.084	0.036
	immediately post-rain	0.806	0.056
	months-after rain	0.41	0.046

especially when maintained in their silk-lined burrows (Rovner 1987). Rovner's report led us to predict that our spiders do not close the entrance of their burrows to avoid the direct contact with rainwater; rather, we think that burrow-plugging could prevent the destruction of the spider's home and the expenditure of energy necessary to its subsequent reconstruction. Indeed, previous observations carried out on *Ariadna* sp. living in areas near Gobabeb (Namibia) (Costa et al. 1993), showed that after a sudden storm in March 1993, several burrow entrances appeared closed with silk and sand and their ring stones scattered. Spiders spent the whole night rearranging their burrow and replacing the stones around the mouth.

The *Ariadna* sp. population of the present study lives in a habitat very rich in lichens. Lichens, an important component of biological soil crusts, act in soil stabilization and in increasing primary production (Lalley & Viles 2005). They play an especially important function in hyperarid areas, such as the Namib Desert, where fog events are regular and sporadic, heavy rainfall occurs (Lalley et al. 2006). Lichen communities are often species-rich and densely crowded with complex biotic interactions (Wessels et al. 1979; Tuba et al. 1998). They provide a milieu that enables the arthropod community to survive extended periods of desiccation within a thallus microhabitat (Seaward 2008). Not surprisingly, in the burrow-surrounding rings of adult *Ariadna* spiders, the number of pieces of lichen exceeds the number of stones, as the correlation analysis indicated. In our opinion, these animals can avoid desiccation due to fog moisture retained by lichens and receive a trophic benefit from lichenophagous prey.

Brief rain pulses, lasting at most several hours, as is characteristic in the Namib Desert, affect the population growth of organisms like soil-dwelling microfauna (Schwinning & Sala 2004). Researchers have also pointed out (Yang et al. 2008) that rain events act as "resource pulses" that are especially important in xeric environments such as deserts, which are described by Noy-Meir (1973) as "pulse-drive ecosystems." Hence, our results lead us to hypothesize that the heavy rain of Mareh 2000 did not pose a real risk for *Ariadna* spider survival and, on the eontrary, that it likely served as a hydrating resource pulse.

Ariadna spiderlings responded to rain differently than adults, possibly because they need to forage frequently. Their burrows are shallow and almost always surrounded only by sand grains; therefore, the rainwater, in addition to the fog moisture, makes sand still more compact, reducing the risk of burrow collapse caused by high winds that characterize the area. Unlike adults, spiderlings cannot survive for long periods without feeding. By opening their small burrows shortly after a rain event, they can benefit from the increased probability of intercepting tiny mites or springtails that live on the soil surface of the Namib Desert (André et al. 1997) and are washed by rainwater into the spider burrows. In other words, we hypothesize that rain pulses can turn into immediate "trophic resource" pulses (Yang et al. 2008) for the *Ariadna* spiderlings.

Finally, "medium size" subadult spiders did not show temporal changes in their burrow opening/closing activity. This perhaps occurred due to a reduced risk of collapse of their burrows and the impelling necessity to gain from the prey the energy required for their molts.

More research is necessary in order to make clear many aspects of the biology of this undescribed *Ariadna* species. Its life cycle is as yet poorly understood. Moreover, we failed to collect any male specimens, so species determination was not possible. Knowing that the Central Namib Desert is a hotspot of endemism for many vertebrates and invertebrates (Simmons et al. 1998; Prendini & Esposito 2010), we suspect that our *Ariadna* sp. might turn out to be another endemic arthropod.

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SHORT COMMUNICATION

Food choice of the Neotropical harvestman *Erginulus clavotibialis* (Opiliones: Laniatores: Cosmetidae)

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Abstract. Relatively little is known about the food habits of neotropical harvestmen. We used *Erginulus clavotibialis* (Pickard-Cambridge 1905), a locally abundant species of cosmetid harvestman in Belize, in a food choice experiment. Individuals were presented with fresh fruit (pineapple) and live invertebrate prey (termites) in an experimental chamber. This species showed a strong preference for fruit, as 72% of individuals ate it first and 67% spent the most time in the fruit-containing portion of the experimental chamber. Five *E. clavotibialis* (13%) consumed termites, confirming this species' ability to capture and consume live invertebrate prey. Adult males located food more quickly than nymphs. Harvestmen feeding on fruit were also significantly more active than non-feeding individuals or those preying upon termites first. Opportunistic frugivory may be important to *E. clavotibialis* during times when fruit is available (e.g., wet season). We hypothesize that this species exhibits a generalist diet in the field.

Keywords: Diet, feeding preference, frugivory, omnivory

Tropical forests have very high biodiversity in a variety of taxa, including terrestrial plants, vertebrates and especially arthropods (Wilson 1992; Myers et al. 2000). Many of these arthropod species have not been formally described (Erwin 1982; Ødegaard 2000), and little is known about the natural history of most species. There are few ecological studies of the harvestman fauna that occurs in the forested habitats of Central America (Proud et al. 2012; Wade et al. 2011). Not surprisingly, then, there is a general lack of detailed information regarding the diet and foraging behavior of these harvestmen (Acosta & Machado 2007).

Harvestmen are typically assumed to be omnivores, consuming live and dead invertebrates, fungi and plant material (i.e., Edgar 1971; Acosta & Machado 2007); with anecdotal observations of large gonyleptids feeding on small frogs (Castanho & Pinto da Rocha 2005) and nestling birds (Benson & Chartier 2010). Several studies have recently revealed that harvestmen are more active predators than previously assumed (i.e., Gnaspini 1996; Halaj & Cady 2000). A few studies have shown evidence of frugivory by harvestmen under laboratory conditions (Capocasale & Bruno-Trezza 1964; Gnaspini 1996) or in the field (Halaj & Cady 2000; Machado & Pizo 2000). However, it remains unclear how common frugivory is among harvestmen or whether fruits are preferred over live invertebrate prey.

The distribution of the harvestman *Erginulus clavotibialis* (Pickard-Cambridge 1905) includes areas in eastern Mexico, Belize and Guatemala, where individuals are relatively abundant in forested habitats (Goodnight & Goodnight 1977). Goodnight & Goodnight (1976) provide descriptions of aspects of the natural history of *E. clavotibialis*, with a primary focus on reproduction and development. This cosmetid species forages nocturnally and is presumed to be omnivorous (Goodnight & Goodnight 1976), but this assertion has not been empirically tested. During our previous field collections, we observed three adult *E. clavotibialis* feeding on a stalk of sugarcane during the evening of 4 January 2012 at the Clarissa Falls Forest reserve (17.1160°N, 89.1198°W). We did not observe any other instances of feeding, but *E. clavotibialis* was often found under the bark of rotting logs or in palm frond sheaths containing termites. We conducted an experimental study of food preference of *E. clavotibialis* using termite prey as a proxy for live invertebrates and pineapple as a proxy for fruit. This allowed us to quantify food choice and feeding habits in an experimental arena and to assess behavioral differences in harvestmen feeding on the different food types.

We collected 39 individuals (22 adult females, 10 adult males and 7 nymphs) by hand from leaf litter, tree buttresses, logs and other debris on 19 July 2012 at the Clarissa Falls resort, Cayo District, Belize (17.1116°N, 89.1272°W). Harvestmen were placed at random into one of three communal housing chambers (2.25L rectangular polypropylene boxes: 14 × 25 × 7.25 cm) lined with leaf litter and bark and kept moist, such that water condensed on the chamber walls. They were not fed for three days between field collection and the feeding experiment, and no mortality was observed between the time of collection and the end of the experiment. At the conclusion of the experiment, all specimens were preserved in 70% ethanol. Voucher specimens were deposited in the collections of the American Museum of Natural History, New York.

For the food choice experiment, each harvestman was placed in the center of experimental chambers identical to the holding chambers, but empty except for 3 small dishes (5.5 cm in diameter), located in the left, right and center thirds of the chamber. The center dish was placed upside down to hold the harvestman prior to testing. One of the side dishes held several small pieces of pineapple (~15 g) and the other held 10–12 live termites (worker caste of *Reticulotermes sp.*). After a 5 min. acclimation period, the center dish was removed and the harvestman was allowed to move around the chamber and feed for 30 min. Harvestmen could move across the chamber in < 10 sec; thus, actively foraging individuals could easily contact and perceive the different food items in the chamber. The trials were conducted after dusk (1900–2400 h) in a darkened room, and harvestmen were observed under red light to minimize disturbance (Hoenen & Gnaspini 1999). In between trials, we removed the harvestmen and the food dishes, replaced food that had been consumed, cleaned the chamber with a paper towel soaked in a 50% isopropyl alcohol solution, allowed chambers to air dry, and randomly reassigned the food dishes among chambers. Each harvestman was tested only once and was then transferred to a separate chamber to hold harvestmen that had completed the experiment.

Seven feeding trials were conducted simultaneously, with two observers each recording data. We recorded the time when a harvestman moved to a different portion of the chamber and when the harvestman fed on one of the food items. We then calculated the proportion of time spent in each section and determined which section each harvestman spent the most time in (preferred area). We also recorded the time until each individual fed upon the first food item and counted the number of termites consumed during each trial. Finally, we tallied the number of times each harvestman moved from one section of the chamber to another, using the total number of

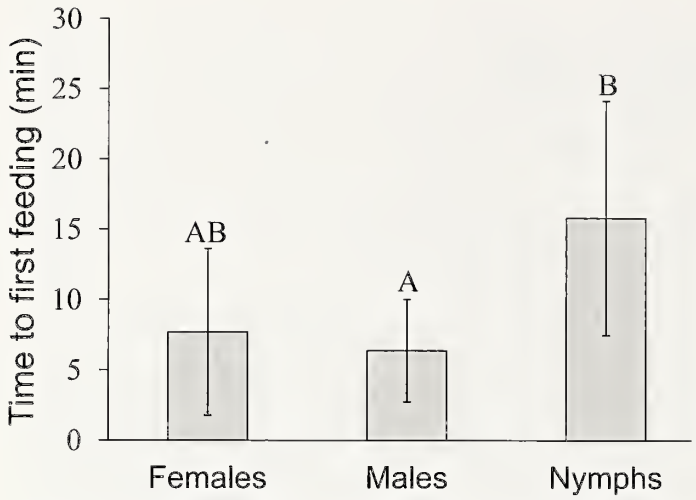

Figure 1.—Comparison of the time to the first feeding for females, males and nymphs. Bars are means, whiskers are SE. Different capital letters indicate significant difference based on the Tukey post hoc test.

sections "visited" as a measure of the frequency of movement. For most comparisons (i.e., food selection, preferred area), we pooled the data, because we did not expect differences in diet between the sexes or between nymphs and adults. However, the males of this species possess a heavily armed leg IV and nymphs are substantially smaller than adults. Thus we hypothesized that movement may vary between males, females and nymphs and compared the number of sections visited and the time to first feeding separately for males, females and nymphs.

Data on feeding preferences and preferred area of the chamber were pooled and analyzed using a G-test for goodness of fit with William's correction (Sokal & Rohlf 1994). The null hypothesis was that there would be no difference in food choice (1:1 ratio between food types) or preferred area (pineapple, termite and control sections would be preferred by an equal number of harvestmen), with the alternative hypothesis that one food type and/or section would be preferred. For females, males and nymphs, we compared the time until the first food item was fed upon and the number of sections visited, using ANOVA with Tukey's post hoc test ($\alpha = 0.05$), because these data met the assumptions of normal distributions and equality of variance. Finally, we compared the number of sections visited by harvestmen that first fed on termites or did not feed with the number of sections visited by harvestmen that first fed on pineapple using a nonparametric Mann-Whitney U test, because the variance was not equal between these groups.

Most harvestmen (28 out of 33 that ate during the experiment) fed upon pineapple first ($G_1 = 17.41$, $P < 0.0001$). Among the five individuals that ate termites first, two later consumed pineapple, and two others consumed additional termites (2–4 total) during the trial. Harvestmen spent ~50% of the time in the pineapple section and ~25% of the time in each of the other sections. The pineapple section was preferred by 26 harvestmen (26 spent the greatest proportion of time there), whereas 8 preferred the termite section and 5 preferred the control section ($G_2 = 18.72$, $P = 0.0001$). Females, males and nymphs did not differ significantly in the number of areas visited ($F_{2,36}=1.66$, $P = 0.20$), but did differ significantly in the time to first feeding ($F_{2,30} = 3.53$, $P = 0.042$), with males feeding significantly faster than nymphs ($P = 0.049$, Tukey's post hoc test; Fig. 1). Harvestmen feeding on pineapple became significantly more active (mean of 10.64 sections visited) than those not feeding on pineapple (average of 3.45 sections visited; $U = 65.5$, $z = 2.75$, $P = 0.006$). We typically observed this increased activity only after the harvestmen finished eating pineapple.

After feeding on pineapple, nearly every individual raised both of the second pair of legs to an almost vertical position and would slowly move them from side to side, often for several minutes at a time. The significance of this behavior was not clear; however, Goodnight & Goodnight (1976) also reported a similar leg-waving behavior in this species, but assumed that it could enhance prey encounter or capture rates. Because many chemosensory organs are located on the second pair of legs, it is possible that this behavior serves some sort of sensory function, but this warrants further study. Likewise, harvestmen have been observed to be attracted to chemical cues released by conspecifics rubbing their bodies on surfaces (Willemart & Hebets 2012). We did not observe this type of scent marking during our experiment, and several aspects of our experimental design (cleaning chambers with ethanol, running several chambers simultaneously, replacing food that had been partly consumed, random reassignment of feeding dishes among experimental chambers) were designed to minimize any potential chemical cues from previous individuals. It is also possible that harvestmen were seeking a food high in water content, and preferred pineapple for this reason. However, *E. clavotibialis* were maintained in a high humidity environment prior to the experiment, and termite body fluids also contain substantial water. Thus, it is more likely that harvestmen were responding to the presence of an aromatic food high in carbohydrates. Other investigators have observed that harvestmen prefer highly aromatic food items (Santos & Gnaspini 2002); thus, future studies could examine the degree to which aroma impacts feeding preferences in the laboratory or field.

In our experiment, *E. clavotibialis* showed a significant preference for fruit in captivity, but also fed upon live invertebrate prey. Frugivory by harvestmen has also been observed under laboratory conditions in other species (Capocasale & Bruno-Trezza 1964; Gnaspini 1996). However, harvestmen diets in the laboratory and field sometimes vary substantially (i.e., Edgar 1971; Gnaspini 1996; Santos & Gnaspini 2002), and thus the implications of these findings for diet in the field warrant further investigation. Likely, the incidence of frugivory will depend on the degree to which fruit sources are available in the field; many tropical forests have abundant sources of seasonably available fruits (Jordano 2000; Machado & Pizo 2000). Different harvestmen species likely vary greatly in their utilization of fruits, but little is currently known about the diets of most tropical harvestmen species (Acosta & Machado 2007). Machado & Pizo (2000) documented frugivory in the field by the gonyleptid *Neosadocus variabilis* (Mello-Leitão 1935), but not by other species occurring at that site in southeastern Brazil. Halaj & Cady (2000) found that sclerosomatid harvestmen (primarily *Leiobunum* spp. Koch 1839) frequently consumed blackberries (up to 25% of the diet) in Ohio, USA, even though invertebrates were more important overall in the diet.

Compared to invertebrate prey, fruits are typically higher in carbohydrates and lower in protein, but this can vary substantially among fruits (Machado & Pizo 2000). Machado & Pizo (2000) observed that the harvestman *N. variabilis* fed on fruits across a wide range of lipid (5.2–70.8%), carbohydrate (16.5–85.5%) and protein (4.6–10.3%) contents, with a marked preference for larger fruits that could not be carried off easily by ants. They suggested that lipid-rich fruits might substitute for a typical diet of insects, and future studies could examine whether harvestmen prefer fruits rich in carbohydrates or lipids and the degree to which frugivory is realized in the field. We hypothesize that *E. clavotibialis* exhibits a generalist diet in the field, utilizing facultative frugivory whenever fruits are abundant.

Some investigators have used stable isotope analysis (Koenig et al. 2011) or polymerase chain reaction (PCR) analysis (Lundgren et al. 2009) to quantify the diets of harvestmen. Stable isotopes of C, N and S are most frequently used for food web analyses to integrate feeding history over time and identify the carbon source and realized trophic level of consumers (Peterson & Fry 1987). Koenig et al. (2011) used N

isotope analysis to document differences in trophic level for the harvestmen *Mitopus morio* (Fabricius 1779). That species showed enrichment in ^{15}N at only one of the sites, indicating that it could function as an intermediate predator or as a top invertebrate carnivore. Lundgren et al. (2009) used PCR analysis on prey DNA extracted from the gut tract of the harvestman *Phalangium opilio* (Linnaeus 1758) to document consumption of the agricultural pest *Diabrotica virgifera*. Future studies could utilize these types of analytical techniques to quantify more fully the diets of harvestmen in the field.

Despite the assumption that harvestmen are generalist omnivores, little is currently known about the diet of most species (Aeosta & Macahdo 2007). Recent studies have provided new insights into the relative importance of carnivory (Gnaspini 1996; Halaj & Cady 2000; Koenig et al. 2011) and frugivory (Gnaspini 1996; Halaj & Cady 2000; Machado & Pizo 2000) in the diets of harvestmen. Facultative frugivory and diet flexibility may enable harvestmen to capitalize on seasonally variable food items when they are abundant. A challenge for future studies will be to identify how flexible the diets of various harvestmen are and the degree to which the conventional broad-based generalizations apply.

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SHORT COMMUNICATION

Meiotic studies in *Brachistosternus alienus* (Scorpiones; Bothriuridae)

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Abstract. *Brachistosternus* Pocock 1893 is the most diverse genus of the scorpion family Bothriuridae. Only four species of the genus have been cytogenetically analyzed so far. We report herein the cytogenetic analysis of *Brachistosternus alienus* Lönnberg 1898 from Comallo (Río Negro province, Argentina). This species is widely distributed in the Monte phytogeographic province, located in central and northern Argentine Patagonia. Meiotic cells of *B. alienus* from Comallo show 23 homomorphic achiasmatic bivalents. The karyotype of this species contains scarce AT-rich regions that may be associated with the heterochromatin of centromeric regions. Giacomozzi (1977) reports $n = 14$ for *B. alienus* from Chubut province. Unfortunately, it is not presently possible to determine if those specimens correspond to *B. alienus* or to a sympatric species, *Brachistosternus angustimanus* Ojanguren-Affilastro & Roig-Alsina 2001. These different chromosome numbers of the two populations analyzed may reflect the occurrence of a chromosomal polytypism in *B. alienus*, or they may characterize different species.

Keywords: Cytogenetics, DAPI staining, karyotype, meiosis, scorpion

In recent years, the aim of our research group has been to study cytogenetics in scorpions belonging to the family Bothriuridae Simon 1880. The genus *Brachistosternus* Pocock 1893 is of particular concern because its species-level taxonomy is fairly well resolved (Rodríguez Gil et al. 2009). Moreover, the phylogeny of this group has been recently evaluated (Ojanguren-Affilastro & Ramírez 2009), providing a more comprehensive evolutionary-based framework for cytogenetic studies.

Brachistosternus is the most diverse genus of the family Bothriuridae, comprising 40 of the ~150 known species of the family. Its species inhabit arid and semi-arid areas of western and southern South America, from Ecuador to southern Patagonia, Argentina (Cekalovic 1969; Ojanguren-Affilastro 2001, 2003a, b, 2005a, b; Ochoa & Ojanguren-Affilastro 2007; Ojanguren-Affilastro & Scioscia 2007; Ojanguren-Affilastro et al. 2007a, b). The group's tremendous diversification is relatively recent, and may have resulted from the onset of aridity in the late Miocene (7–10 mya); most of the basal bothriurid genera occur in mesic environments (Prendini 2003).

To date, there are few published cytogenetic studies on bothriurids, comprising only ten species (Piza de Toledo 1947; Ferreira 1968; Giacomozzi 1977; Rodríguez Gil et al. 2009; Schneider et al. 2009a). Four of these species belong to the Argentine representatives of the genus *Brachistosternus*. *Brachistosternus (Ministernus) ferrugineus* (Thorell 1876) and *Brachistosternus* (*Brachistosternus*) *montanus* Roig Alsina 1977 have $n = 23$ (Rodríguez Gil et al. 2009). *Brachistosternus* (*B.*) *pentheri* Mello-Leitão 1931 shows two different cytotypes, $n = 23$ and $n = 21$, which correspond to two slightly different morphs (Rodríguez Gil et al. 2009). Finally, the haploid karyotype of *Brachistosternus* (*B.*) *alienus* Lönnberg 1898 is reported to consist of 14 chromosomes (Giacomozzi 1977).

Brachistosternus alienus is widely distributed in the Monte phytogeographic province, located in central and northern Argentine Patagonia. A taxonomic revision of this species (Ojanguren-Affilastro 2001) revealed that geographically distant populations exhibit some morphological differences, raising doubts about their identity. There are no data on the cytogenetics of this species except for the chromosome number of specimens from the Patagonian province of Chubut (Giacomozzi 1977); however, this information is incomplete and species identity of these specimens is uncertain.

In this study, two males of *B. alienus* belonging to a population from a locality near Comallo (Río Negro province) in northern Argentine Patagonia (41°03′01″S, 70°26′51.7″W) were examined. Comallo is located about 500 km from the type locality in Chubut province. The karyotype and meiotic behavior of chromosomes was described and furthermore, the distribution of the AT-rich regions in a representative of the genus *Brachistosternus* was determined for the first time.

The males of *B. alienus* were collected at night using UV lamps, then were carried to the laboratory and killed by cooling to −20°C. Their gonads were dissected in saline solution (0.154 M NaCl), incubated in hypotonic solution (1:1 saline solution:distilled water, 30 min), fixed in a freshly prepared mixture of ethanol:chloroform:acetic acid (6:3:1, 30 min), and stored in fresh fixative mixture. Pieces of testis were placed on slides and dissociated in a drop of 80% acetic acid with tungsten needles. Preparations with a drop of suspension were placed on a heated histological plate (approximately 45°C); the suspension was spread on the slides using a tungsten needle. Finally, the preparations were air-dried and stained with 5% Giemsa solution in distilled water for 10 min. Following light microscopy observations, the slides were destained using a mixture of ethanol:acetic acid (3:1) for 2 h and stained with fluorescent dye DAPI (4′-6-diamidino-2-phenylindole) to detect blocks of AT-rich regions. Briefly, the slides were rinsed twice with 4xSSC buffer for 10 min and air-dried. One drop of 0.5 µg/ml DAPI in PBS (phosphate buffered saline) containing 1% Triton X-100 was placed on each slide, covered with a coverslip and incubated in a moist chamber (30 min, room temperature). Following coverslip removal, the slides were rinsed with 4xSSC and air-dried. Finally, they were mounted with 50 µl of VECTASHIELD® (Vector Laboratories, Inc.), covered by a coverslip, and stored at 4°C overnight before microscopic analysis. The staining with DAPI and air-drying steps were performed in the dark.

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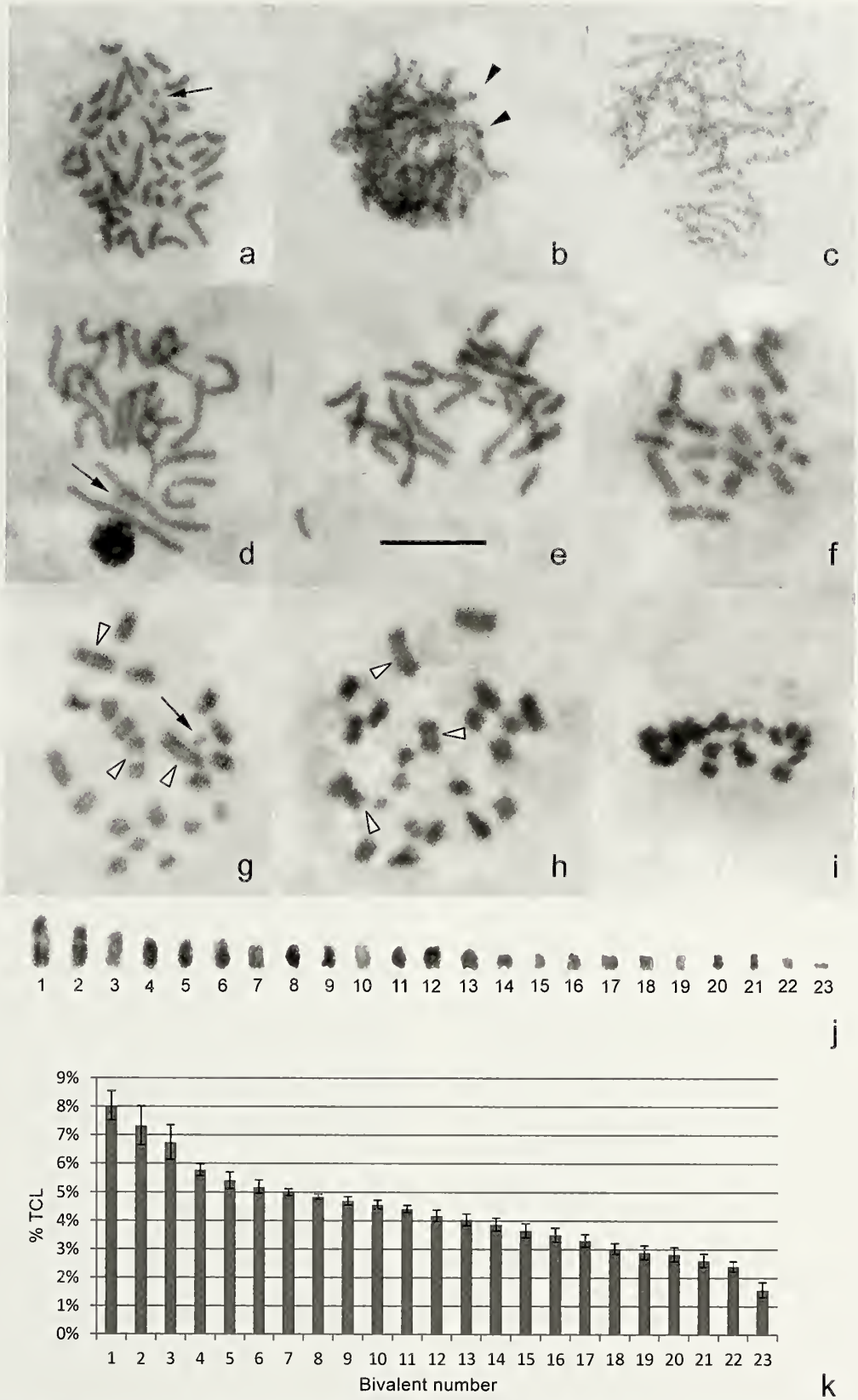

Figure 1.—Spermatogenesis, meiotic karyogram and ideogram of *Brachistosternus alienus* ($2n = 46$, $n = 23$). a. Early spermatogonial metaphase; b. Zygotene; c. Pachytene; d. Early postpachytene; e. Middle postpachytene; f. Late postpachytene; g,h. Prometaphase I; i. Metaphase I; j. Meiotic karyogram (based on prometaphase bivalents depicted in 1g); k. Meiotic idiogram. The arrows point to the smallest pair. The black arrowheads point to the positively heteropycnotic telomeric regions. The white arrowheads mark the increase in separation of the homologous chromosomes. Scale = 10 μm.

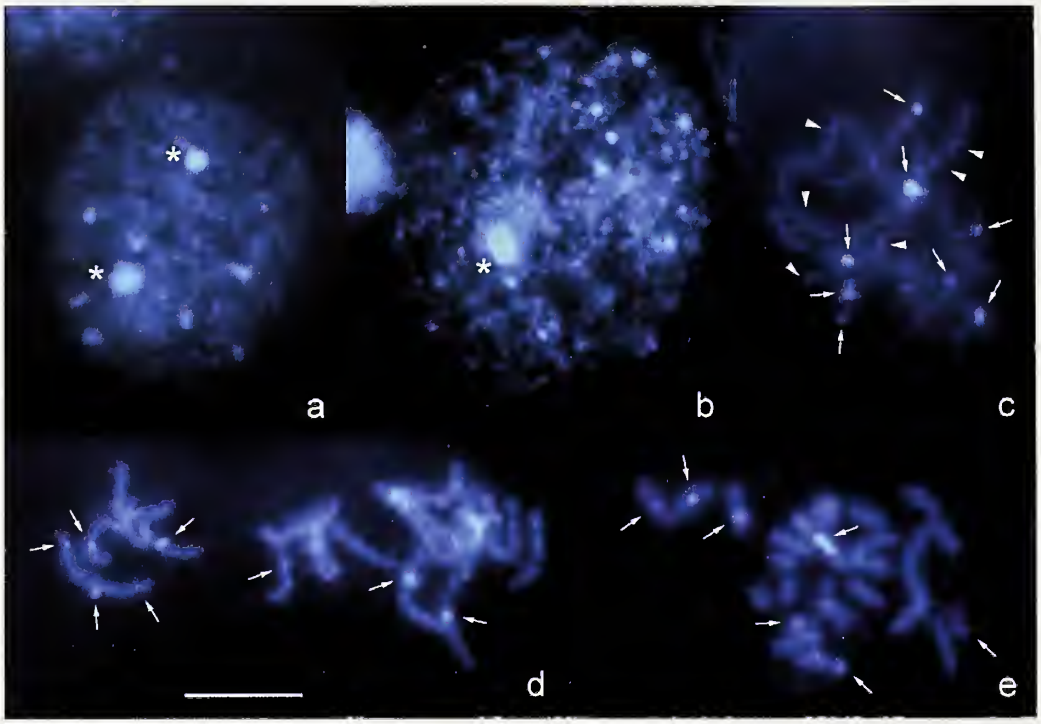

Figure 2.—DAPI staining of interphase nuclei and meiotic chromosomes of *Brachistosternus alienus*. a. Interphase; b. Leptotene-zygotene; c. Late pachytene; d. Early postpachytene; e. Late postpachytene. Asterisks point to chromocenters. Arrows point to DAPI-positive bands showing higher intensity of fluorescence. Arrowheads point to DAPI-positive bands exhibiting lower intensity of fluorescence. Scale = 10 μm.

To determine the meiotic karyotype, chromosome measurements were performed in 11 well-spread postpachytene and prometaphase I using Micro-Measure software, version 3.3 (Reeves & Tear 2000). The relative length of each bivalent was calculated as a percentage of total complement length (TCL; length of all bivalents of the karyotype). These data allowed us to construct an ideogram. Bivalents of seven prometaphase I exhibiting the same degree of condensation were measured to determine absolute length (μm) of each bivalent.

The spermatogonial mitosis shows 46 monocentric chromosomes; one pair is remarkable for its small size (Fig. 1a). During zygotene, terminal regions of some bivalents exhibit small positively heteropycnotic blocks (Fig. 1b), which are no longer distinguishable at pachytene (Fig. 1c). Twenty-three bivalents are seen from late pachytene until prometaphase I (Fig. 1d–h). During the chromatin condensation, at early and middle postpachytene the homologous chromosomes of the bivalents remain tightly joined (Fig. 1d, e). At late postpachytene it can be seen that the homologous chromosomes lie parallel to each other, thus showing the absence of chiasmata, which is a common feature of meiosis in male scorpions (Fig. 1f). At prometaphase I and metaphase I, many bivalents show a small region in the intercalary or terminal position where the homologues are more separated (Fig. 1g–i).

The meiotic karyotype shows 23 homomorphic bivalents (Fig. 1j). Three groups of bivalents can be identified as follows: three large bivalents of different size (4.58, 4.08 and 3.73 μm), 19 middle-sized bivalents decreasing gradually in size (3.30, 3.13, 3.02, 2.92, 2.83, 2.73, 2.66, 2.59, 2.45, 2.38, 2.28, 2.16, 2.08, 1.95, 1.79, 1.76, 1.73, 1.57 and 1.44 μm), and the smallest bivalent of the complement (0.94 μm). The relative length of the bivalents ranges from 8.03% to 6.74% in the first group, from 5.77% to 2.41% in the second group, and is 1.59% for the smallest bivalent (Fig. 1k).

Fluorescent banding using DAPI revealed AT-rich regions, namely chromocenters at interphase nuclei as well as bands on bivalents at prophase of the first meiotie division. These regions vary in size and number. They are presumably formed by AT-rich constitutive heterochromatin. Interphase and leptotene/zygotene nuclei usually contain one or two conspicuous DAPI-positive chromocenters as well as some signals of intermediate or low intensity (Fig. 2a, b). Pachytene karyotypes exhibit between eight and 10 intercalary DAPI-positive bands showing higher intensity of fluorescence and some bands of lower intensity in terminal or intercalary regions (Fig. 2c). During the following bivalent condensation, most of the low-intensity bands become indistinguishable (Fig. 2d). At late postpachytene, intercalary AT-rich bands can be identified in one large and four middle-sized bivalents, while terminal bands are detected in two middle-sized bivalents (Fig. 2e).

Heterochromatin distribution has been analyzed in only a few scorpions so far, namely eight species of Buthidae (belonging to genera *Isometroides*, *Isometrus*, *Lychas* and *Tityus*) (Shanahan 1989a; Schneider et al. 2009b; Schneider & Cella 2010), six species of Urodacidae (genus *Urodacus*) (Shanahan 1989b), and two species of Bothriuridae belonging to the genus *Bothriurus* (Schneider et al. 2009a). Heterochromatin content and distribution in Buthidae is variable. In contrast to the other scorpions, this family shows holokinetic chromosomes. Most heterochromatin of buthids occurs at telomeric regions. Shanahan (1989b) reports pericentromeric bands in most *Urodacus* species studied. In *Bothriurus rochensis* Schneider et al. (2009a) report small C-bands in the centromeric regions of some subtelocentric and submetacentric pairs, as well as in the terminal region of the long arm of several submetacentric pairs. These authors also described AT-rich bands in the terminal regions of two pachytene bivalents of *B. rochensis*. In contrast, heterochromatin has not been detected by C-banding and AT specific fluorochrome in *Bothriurus araguayae* (Schneider et al. 2009a). Blocks of presumed AT-rich heterochromatin of *Brachistosternus alienus* may correspond to pericentromeric regions of monobrachial and bibrachial chromosomes.

For *Brachistosternus* species evaluated so far, the haploid set of *B. ferrugineus* and *B. montanus* is formed by 23 chromosomes. The same number is found in populations of *B. pentheri* from the northernmost

limit of the species distribution (north of La Rioja province, Argentina) (Rodríguez Gil et al. 2009). Individuals of *B. pentheri* from northern populations are larger and much less pigmented than the typical morph. On the other hand, populations corresponding to the holotypic morph, which are distributed from the south of La Rioja province to the southeast of Buenos Aires province, have n = 21 (Giacomozzi 1977; Rodríguez Gil et al. 2009). Rodríguez Gil et al. (2009) propose that marginal populations of *B. pentheri* from northern Argentina could be considered a subspecies or even a different species due to their specific morphological features and different chromosome number.

The haploid karyotype of *B. alienus* from Comallo (province of Río Negro, Argentina) (this study) comprises 23 chromosomes, whereas the specimens of *B. alienus* studied by Giacomozzi (1977) have n = 14. Giacomozzi (1977) stated that the specimens were determined by Dr. E. Maury and sampled in the province of Chubut, Argentina. Although further information on the collection site is missing, data from that period suggests that the specimens were collected near Puerto Madryn, the species' type locality. However, it is impossible to determine if the specimens studied by Giacomozzi (1977) belong to *B. alienus* or to *B. angustimanus* Ojanguren-Affilastro & Roig-Alsina 2001 (a sympatric species), because the material is no longer available. At the time of Giacomozzi's study, most authors based the identification of *B. alienus* on a redescription of this species by Mello-Leitão (1934, 1945), which corresponds more closely to *B. angustimanus* than to the original description of *B. alienus* by Lönnberg (1898). Both species are sympatric over most of their geographic range. *Brachistosternus angustimanus* is larger and usually more abundant than *B. alienus* (Ojanguren-Affilastro 2001; Ojanguren-Affilastro & Roig-Alsina 2001; Rodríguez Gil et al. 2009). Conclusive evidence of species misidentification was provided by Maury (1972), who assigned a hemispermatophore of *B. angustimanus* to *B. alienus*.

The considerations mentioned above and the reduced chromosome number (n = 14) of specimens studied by Giacomozzi suggest that these specimens belonged to *B. angustimanus*. However, we cannot rule out the possibility that Giacomozzi's specimens correspond to *B. alienus*; in this case, species should be considered polytypic, like *B. pentheri* (Rodríguez Gil et al. 2009). The specimens analyzed in this work vary slightly in morphology from the typical morph, and Comallo is about 500 km from the type locality of *B. alienus* (the putative sampling site of Giacomozzi's specimens) (Ojanguren-Affilastro 2001, 2005b). At this time access to living material of either *B. alienus* or *B. angustimanus* from Puerto Madryn for cytogenetic analysis was not possible. Further studies involving individuals unequivocally identified as *B. alienus* and *B. angustimanus* from different localities are needed to establish whether variation in chromosome number reflects the occurrence of polytypism or different species.

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